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COMPARATIVE INHALATION SCREEN OF TITANIUM DIOXIDE AND GRAPHITE DUSTS

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Occupational exposure to airborne graphite may occur in manufacturing and application processes. Synthetic graphite is a pure crystalline form of carbon produced from high temperature treatment of petroleum products and contains less than 1% quartz; it is considered a "nuisance" dust by the American Conference of Governmental Industrial Hygienists. Natural graphite is the mineral form of graphitic carbon and contains associated silicate minerals. Titanium dioxide is also regarded as a "nuisance" dust and was used as a negative control in this study. Fischer 344 rats were exposed via whole body inhalation to 100 mg/m³ of synthetic graphite, natural graphite, and titanium dioxide for 4 hr/day for 4 days. At 24 hr and 14 days post-exposure (PE), exposed and air exposed controls were evaluated for bronchoalveolar lavage (BAL), physiological, and pathological changes. BAL changes were the most sensitive indicator of damage;

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19. ABSTRACT (continued)

although the enzymatic and cytological alterations were evident with all three materials, there were greater increases with the graphite dusts. By 14 days PE, all BAL changes were resolved. There appears to be no deleterious tissue reaction to any of the materials at the levels tested in this study. (AW)

PREFACE

The work described in this report was funded by U.S. Army Medical Research and Development Command under Project Order 87PP7870. This work was started in September 1987 and completed in June 1988.

The use of trade names or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the committee on Revision of the Guide of Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

This report has been approved for release to the public.

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

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SAT Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

SAT In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

____For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

Sandra A. Thomas 28 April 88

PI Signature Date

QUALITY ASSURANCE

This study was examined for compliance with Good Laboratory Practices as published by the U. S. Environmental Protection Agency in 40 CFR Part 792. The dates of all inspections and the dates the results of those inspections were reported to the Study Director and management were as follows:

Phase inspected:	Date:	Date reported:
Pre-exposure animal weighing	11 Dec 87	11 Dec 87
Dosing (via inhalation)	14 Dec 87	15 Dec 87
Necropsy and physiology	17 Dec 87	18 Dec 87
Contractor pathology report	24 Mar 88	24 Mar 88
Data	10 May 88	10 May 88
Final report	10 May 88	10 May 88

To the best of my knowledge, the methods described were the methods followed during the study. The report was determined to be an accurate reflection of the data obtained.

Dennis W. Johnson 12 May 88

Dennis W. Johnson
Director, Quality Assurance Unit
Toxicology Division

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COMPARATIVE INHALATION SCREEN OF TITANIUM DIOXIDE AND GRAPHITE DUSTS

1. INTRODUCTION

Synthetic and/or natural graphite dust may have military applications which could result in inhalation hazards. CRDEC has tested synthetic (Asbury Micro 260) and natural (Asbury Micro 650) graphites and found that acute inhalation exposure in Fischer 344 rats resulted in a mild reversible inflammatory response at high concentrations (500 mg/m^3) for the synthetic material.(1) A repeated inhalation study with the synthetic graphite also showed more changes at a lower concentration (100 mg/m^3) reversible at 3 months post-exposure (PE).(2) The purpose of this study was to compare the toxicity of natural and synthetic graphite using titanium dioxide as a negative control. Both synthetic graphite and titanium dioxide are classifed as "nuisance dusts" as defined by ACGIH.(3) Purported "nuisance" dusts have a history of little adverse effect and do not produce significant organic disease or toxic effect when exposures are kept under control. A Threshold Limit Value (TLV) of 10 mg/m^3 of total dust (less than 1% quartz) is recommended for "nuisance" dusts for a normal workday. For materials containing more than 1% quartz, the environment should be evaluated against the TLV of 0.1 mg/m^3 for respirable quartz. The natural graphite used in this study contains 1.85% silica and chemically may not meet the nuisance dust requirement; however, this material may behave biologically like other nuisance dusts (synthetic graphite and titanium dioxide). According to the ACIGH, the biological criteria of a nuisance dust is defined by the following lung tissue reaction: 1) the architecture of the air spaces remains intact; 2) collagen (scar tissue) is not formed to a significant extent; and 3) the tissue reaction is potentially reversible. In addition to these histopathological indicators of toxicity, pulmonary function and bronchoalveolar lavage (BAL) were used to compare the toxicity of these graphite dusts to titanium dioxide.

2. MATERIALS AND METHODS

2.1 Experimental Design and Test Materials

Groups of 20 male Fischer 344 rats were exposed by whole body inhalation to 100 mg/m^3 of each test material on four consecutive days, four hours/day according to the protocol (see appendix A). Additional groups of 12 rats were also exposed and evaluated for pulmonary function and BAL changes. Microscopic evaluation of the respiratory tract and major organs was done at two time periods PE, 24 hours and 14 days according to the following schedule:

Material	Number of Rats			
	24hr & 14da Path		24hr & 14da BAL/Physio	
Synthetic Graphite	10	10	6	6
Natural Graphite	10	10	6	6
Titanium Dioxide	10	10	6	6
Control	10	10	6	6

The synthetic graphite used in this study is Asbury Micro 260 (less than 1% silica) and the natural graphite is Asbury Micro 650 (1.85% silica). The titanium dioxide was a gift from NL Chemicals Inc. and is a high purity rutile form of titanium dioxide. All three test materials contained neglible amounts of contaminants (see appendix A).

2.2 Chamber Operation

The Hazelton 2000 liter stainless steel inhalation chambers were used for this study. These are "live in" chambers designed to house test animals during the entire course of the study. It incorporates compartmental cage units mounted at six different vertical heights as shown in figure 1. A unique feature of the chamber is the multi-tier arrangement of the cage units and catch pans which facilitates good mixing within the chamber and helps promote a nearly uniform aerosol concentration throughout the chamber. (4) This uniformity has been verified by both fixed point aerosol sampling measurements, residence time distribution measurements, and flow visualization studies. (5,6)

Four Hazelton 2000 liter chambers were set up as shown in figure 2 in building E3266 which is climate controlled (temperature = 74° ± 4°; relative humidity = 40% ± 10%). All four chambers are manifolded to a single blower unit which pulls air from the surrounding room through each of the chambers; all air is filtered prior to being exhausted outside. Chambers designated #2, #3, #4 are set up to handle the various aerosol exposure atmospheres and the inlet ports to these chambers are each fitted with 15 liter glass mixing bowls which aid in aerosol dispersal. Chamber #1 has been designated as the control chamber and is fitted with a particulate filter on its input in place of a mixing bowl. Each chamber exhaust line has an orifice meter downstream of the particulate filter for the purpose of monitoring chamber flow. Gate valves provided on each chamber enable course flow regulation.

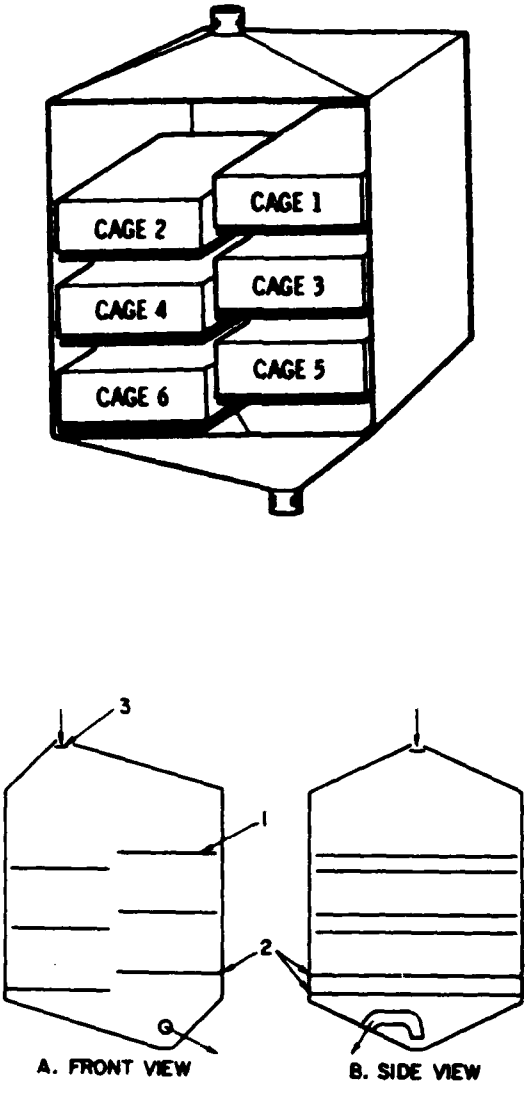


FIGURE 1. Hazelton Chamber System

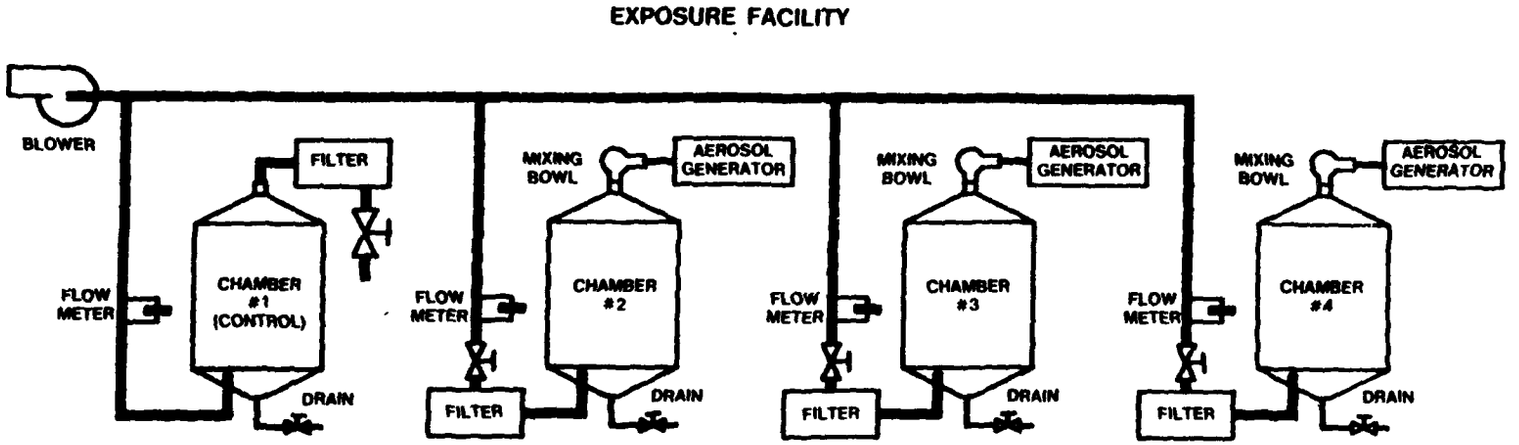


Figure 2. Hazelton 2000 Liter Chambers

The aerosol generation system for titanium dioxide consisted of an AccuRate series 300 screw feeder and attached vibration device which metered the dust at a uniform rate to a Jet-O-Mizer aerosol mill depicted in figure 3. The aerosol mill was equipped with air jets supplied with compressed air at 55 psig. High velocity air emanating from the jets resulted in high particle to particle shear forces which readily caused the break up of agglomerates. Consequently, a relatively highly dispersed aerosol was produced at the outlet of the aerosol mill.

The AccuRate series 300 feeders were also used to deliver the graphite dusts to the dust generators. Dispersion of the graphite dusts was accomplished using a Metronics aerosol generator depicted in figure 4. This device is in essence a centrifugal blower with a deep bladed impellor. Feed material falls into the center of the impellor and is driven against the blades by centrifugal force resulting in particle deagglomeration and dispersion. The resultant aerosol was fed directly into the chamber mixing bowl. The appropriate blower speed was determined during the calibration phase and was regulated by means of a variac.

Each chamber was equipped with a Magnehelic pressure gauge (range 0 to 2" water) to assess internal chamber pressure. Chamber pressures must be maintained at a pressure of negative 0.4 inches of water relative to the surrounding room at all times during an exposure to prevent leakage of chamber atmosphere into the room. Each chamber was also equipped with a platinum resistance thermometer for continuous temperature monitoring during the exposure. The control chamber was equipped with an electrical resistance hygrometer for continuous humidity monitoring. The output from this device was taken as being representative of all four chambers since the probe connot be placed into a dusty atmosphere without resultant damage. Each chamber orifice meter was equipped with a differential pressure transducer for continuous flow monitoring. Chamber flow, temperature, and relative humidity were continuously recorded during the course of the exposure operation.

2.3 Chamber Calibration

The purpose of the calibration phase of the experiment was to quantitate the uniformity and stability of the aerosol mass concentration within the exposure chambers prior to the actual animal exposure. To accomplish this, a four hour mock exposure test was conducted on two separate occasions with each of the three exposure materials (natural graphite, synthetic graphite, and titanium dioxide). Simultaneous filter samples taken at five different sampling

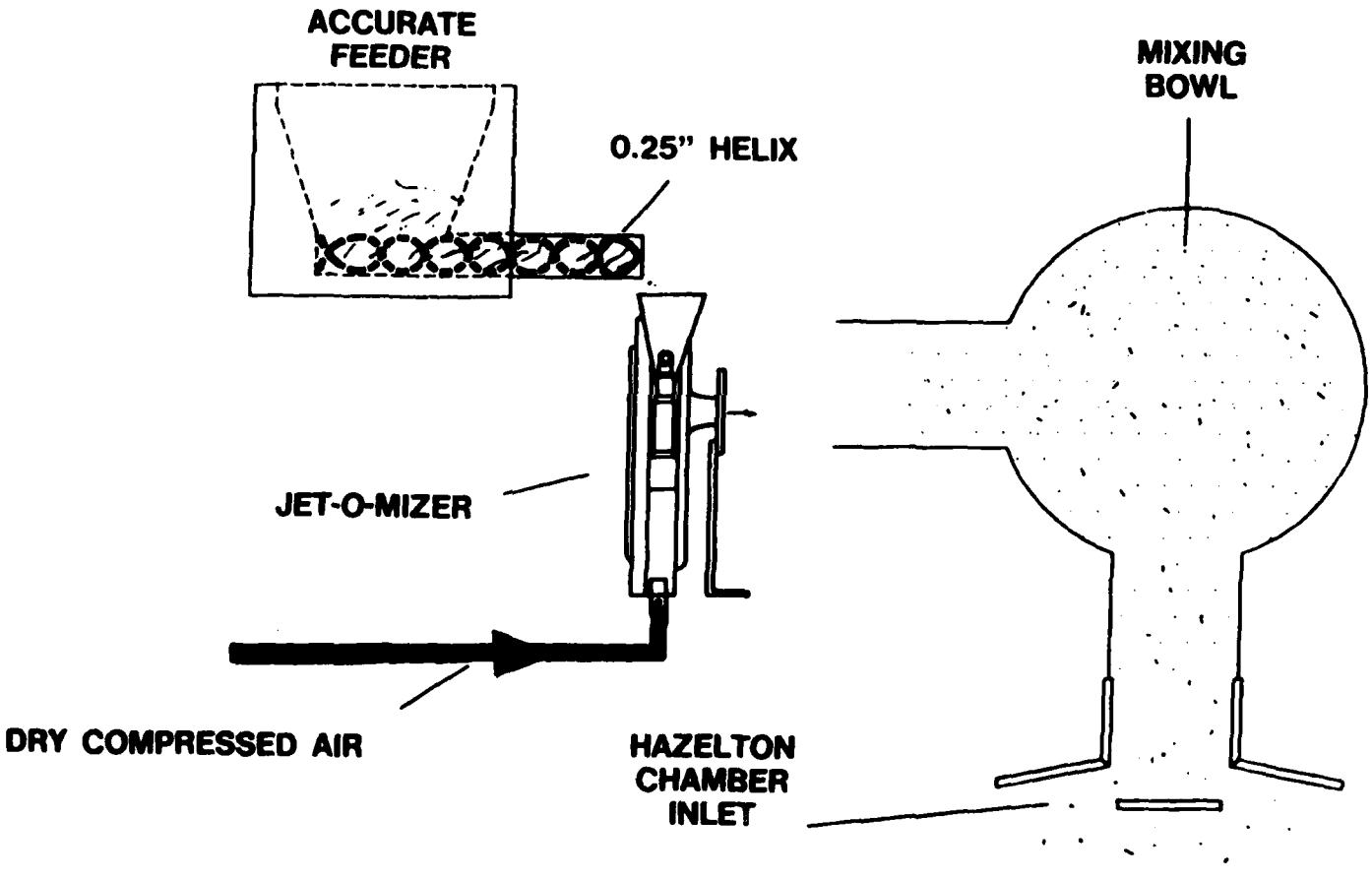


Figure 3. TiO_2 Aerosol Generation System

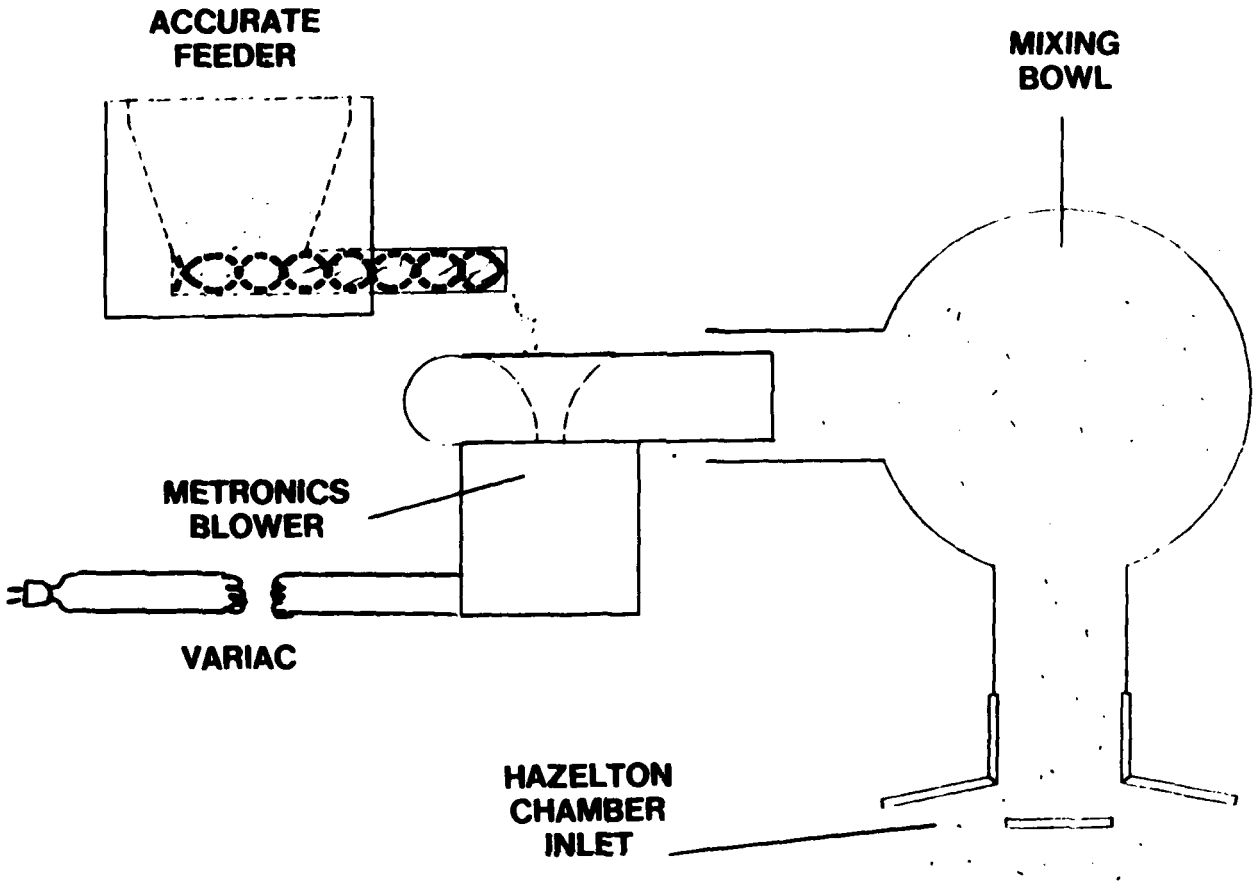
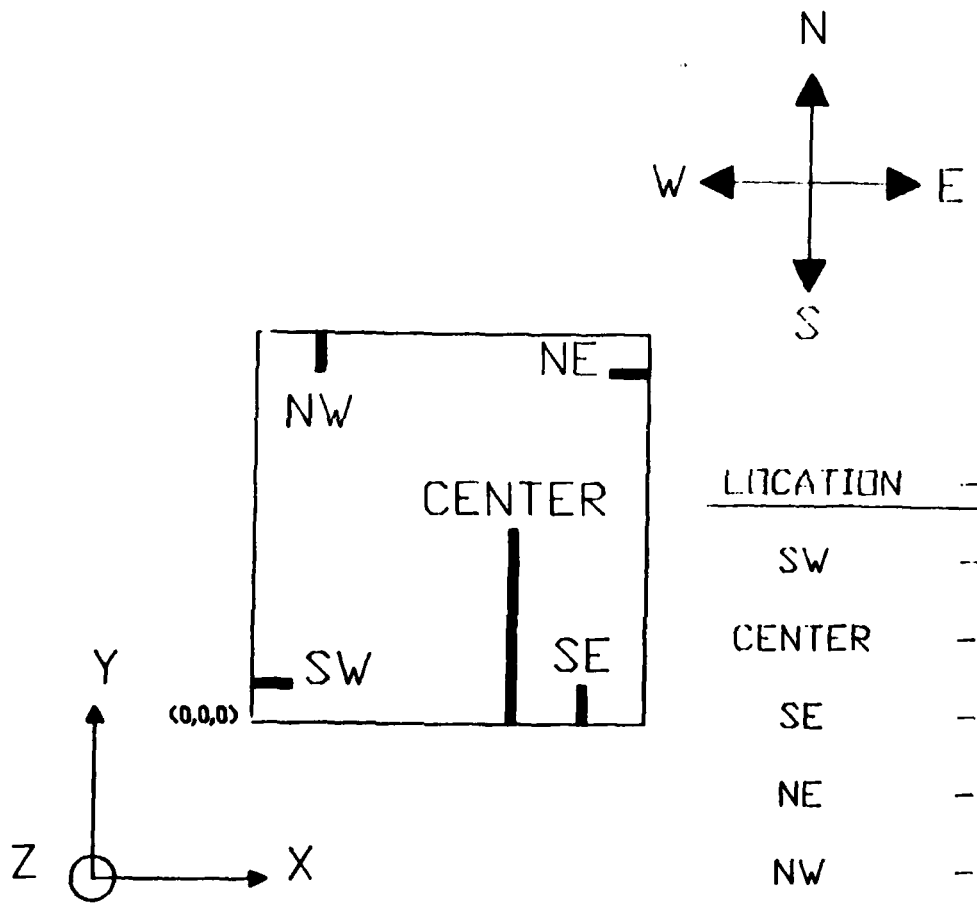


Figure 4. Graphite Aerosol Generation System

positions within the chamber were weighed to determine the aerosol concentrations at these locations. These sampling locations are just above the animal cages in the middle tier of the chambers and lie in a horizontal plane. The sampling locations are defined in figure 5 by number, designated chamber corner, and 3-D coordinates. Sampler location #2 is near the "center" of the chamber. During the course of a mock exposure, five or more sets of filter samples were taken. The flow rate for each sampler was set at 5 lpm and the sampling duration was 5 minutes. The more than 50 filter samples taken per chamber during the two calibration runs were used to evaluate the spatial and temporal variations in aerosol concentrations within the chambers.

The exposure chamber concentration measurements for the calibration phase are shown in Tables 1, 2, and 3 for the natural graphite, synthetic graphite, and titanium dioxide aerosols, respectively. The central columns of data show the aerosol mass concentrations (mg/m^3) taken simultaneously at the five sampler locations for consecutive times (shown above each column) during the course of a run. The block of numbers to the right of the central group are the run statistics computed horizontally for each sampler location. They represent from left to right the temporal average, standard deviation, coefficient of variation, and percentage variation from the mean for the concentration measurements at a given sampler location. Correspondingly, the block of numbers below the central group are the run statistics computed vertically at each elapsed time, and describe the spatial variation in aerosol concentration within the chamber at a particular time. Table 4 summarizes the temporal and spatial distribution for the three aerosols. The overall coefficients of variation represent an average of the individual values computed during the course of the two calibration runs on each material. The mean concentration value shown is the average of all samples taken during the calibration from each chamber. The data indicate that the overall temporal variation at any one location was less than 10 percent on the average. Overall spatial variation achieved was less than 5 percent on average. A one way analysis of variance on the pooled data from the two calibration runs indicated that the differences in measured aerosol concentrations at the five chamber locations at any one time were not significant ($p < 0.05$). The calculations for the latter are shown in more detail in Appendix B.

During the four days of animal exposure, all aerosol filter samples were taken from each chamber at port #4 located at the northeast (NE) corner of the chambers. This was considered to give an adequate estimate of chamber concentration in view of the calibration results and also enabled more frequent sampling from all three chambers during the course of the exposure. A minimum of two samples per hour were taken from each chamber.



LOCATION	- (X,	Y,	Z)
SW	- (6",	11",	30",)
CENTER	- (30",	25",	28",)
SE	- (45',	6",	28',)
NE	- (44',	38",	28",)
NW	- (5',	43",	31",)

FIGURE 5. Location of Aerosol Samplers Top View of Chamber

TABLE 1. EXPOSURE CHAMBER CALIBRATION DATA FOR NATURAL GRAPHITE

CALIBRATION: Natural Graphite Run#1
DATE: 10/20/87

	Elapsed Time, hours											
	0.5	1	2.8	3.25	3.7	4.3	4.8	5.5	Time			
Sampler					3				Average	Std	C.V.	% Var
Location	Concentration, mg/m											
SW	102	91.5	116	118	109	100	94.5	116	105.9	10.3	9.7%	13.6%
CENTER	94	87	110	116	105	97.2	90.4	108	101	10.3	10.2%	14.9%
SE	99.9	91	114	119	112	101	94.1	112	105.4	10.2	9.7%	13.7%
NE	97.2	88	110	109	109	95	89.4	110	101	9.6	9.5%	12.9%
NW	92.1	82	107	110	99	96	87	102	96.9	9.6	9.9%	15.4%
Spatial Average	97	87.9	111.4	114.4	106.8	97.8	91.1	109.6				
Std	4.1	3.8	3.6	4.6	5	2.6	3.2	5.2				
% Var.	5.0%	7.0%	4.0%	5.0%	7.0%	3.0%	5.0%	7.0%				
C.V.	4.2%	4.3%	3.2%	4.0%	4.7%	2.7%	3.5%	4.7%				

CALIBRATION: Natural Graphite Run#2
DATE: 10/26/87

	Elapsed Time, hours										
	0.4	1.2	2.2	3.2	3.9	4.2		Time			
Sampler					3			Average	Std	C.V.	% Var
Location	Concentration, mg/m										
SW	89.2	96.5	96.9	106	90.2	112		98.5	8.9	9.0%	13.7%
CENTER	85.6	91.1	93.7	101	90.1	107		94.8	7.9	8.3%	12.9%
SE	87.6	95.4	95.7	105	90	112		97.6	9.3	9.5%	14.8%
NE	87	89.3	95.7	104	88.1	107		95.2	8.6	9.0%	12.4%
NW	80.9	92.4	94.1	102	85.3	108		93.8	10.1	10.8%	15.1%
Spatial Average	86.1	92.9	95.2	103.6	88.7	109.2					
Std	3.2	3	1.3	2.1	2.1	2.6					
% Var.	6.0%	4.0%	2.0%	3.0%	4.0%	3.0%					
C.V.	3.7%	3.2%	1.4%	2.0%	2.4%	2.4%					

TABLE 2. EXPOSURE CHAMBER CALIBRATION DATA FOR SYNTHETIC GRAPHITE

CALIBRATION: Synthetic Graphite Run#1
DATE: 11/3/87

	Elapsed Time, hours						Time			
	0.2	0.7	1.25	2	3	3.75				
Sampler Location	Concentration, mg/m3						Average	Std	C.V.	% Var
SW	103	107	114	102	95.6	109	105.1	6.4	6.1%	9.0%
CENTER	101	107	114	101	93.4	105	103.6	6.9	6.7%	10.0%
SE	101	106	113	102	93.2	108	103.9	6.8	6.5%	10.3%
NE	98.8	105	113	104	96.2	110	104.5	6.4	6.1%	8.1%
NW	99.7	101	111	101	93	107	102.1	6.2	6.1%	8.9%
Spatial Average	100.7	105.2	113	102	94.3	107.8				
Std	1.6	2.5	1.2	1.2	1.5	1.9				
% Var.	2.0%	4.0%	2.0%	2.0%	2.0%	3.0%				
C.V.	1.6%	2.4%	1.1%	1.2%	1.6%	1.8%				

CALIBRATION: Synthetic Graphite Run #2
DATE: 11/5/87

	Elapsed Time, hours							Time			
	0.7	1.2	2	2.7	3.5	4	4.5				
Sampler Location	Concentration, mg/m3							Average	Std	C.V.	% Var
SW	95.8	106	123	90.4	113	110	117	107.9	11.6	10.8%	16.2%
CENTER	91.6	105	124	90.6	113	106	114	106.3	12.1	11.4%	16.7%
SE	92.9	102	122	87.5	109	107	112	104.6	11.7	11.2%	16.6%
NE	93.7	102	120	89.3	112	108	114	105.6	11.1	10.5%	15.4%
NW	89.3	107	123	89.3	111	107	113	105.7	12.4	11.7%	16.4%
Spatial Average	92.6	104.4	122.4	89.4	111.6	107.6	114				
Std	2.4	2.3	1.5	1.2	1.7	1.5	1.9				
% Var.	4.0%	2.0%	2.0%	2.0%	2.0%	2.0%	3.0%				
C.V.	2.6%	2.2%	1.2%	1.3%	1.5%	1.4%	1.7%				

TABLE 3. EXPOSURE CHAMBER CALIBRATION DATA FOR TITANIUM DIOXIDE

CALIBRATION: Titanium Dioxide Run #1
DATE: 11/25/87

	Elapsed Time, hours					Time			
	0.2	1	2	3	4				
Sampler Location	Concentration, mg/m 3					Average	Std	C.V.	% Var
SW	100	103	103	119	102	105.4	7.7	7.3%	12.9%
CENTER	106	110	109	115	110	110	3.2	2.9%	4.5%
SE	92.8	96.2	102	114	96.6	100.3	8.3	8.3%	13.7%
NE	104	107	96.3	117	109	106.7	7.5	7.0%	9.7%
NW	101	107	104	114	104	106	4.9	4.6%	7.5%
Spatial Average	100.8	104.6	102.9	115.8	104.3				
Std	5	5.3	4.5	2.2	5.5				
% Var.	8.0%	8.0%	6.0%	3.0%	7.0%				
C.V.	5.0%	5.1%	4.4%	1.9%	5.3%				

CALIBRATION: Titanium Dioxide Run #2
DATE: 12/4/87

	Elapsed Time, hours						Time			
	0.5	1.1	1.8	2.4	3	4				
Sampler Location	Concentration, mg/m 3						Average	Std	C.V.	% Var
SW	90.1	77.8	108		113	94.1	96.6	14.1	14.6%	19.5%
CENTER	85.6	75.7	110	94.1	107	88.2	93.4	13.1	14.0%	19.0%
SE	86.6	73.4	107	85.8	102	79.3	89	13	14.6%	20.2%
NE	93.9	80.4	103	97	112	91.6	96.3	10.7	11.1%	16.5%
NW	90.8	80	111	98.6	112	92.7	97.5	12.4	12.7%	17.9%
Spatial Average	89.4	77.5	107.8	93.9	109.2	89.2				
Std	3.4	2.9	3.1	5.7	4.7	5.9				
% Var.	5.0%	5.0%	4.0%	9.0%	7.0%	11.0%				
C.V.	3.8%	3.7%	2.9%	6.1%	4.3%	6.6%				

TABLE 4. SUMMARY OF CALIBRATION DATA

EXPOSURE CHAMBER PERFORMANCE EXPRESSED AS THE MEAN +/- THE STANDARD DEVIATION (RANGE) OF THE COEFFICIENT OF VARIATION (C.V.) FOR SPATIAL AND TEMPORAL VARIATIONS IN AEROSOL CONCENTRATION

Measurement	Chamber #2 Natural Graphite	Chamber #3 Synthetic Graphite	Chamber #4 Titanium Dioxide
Overall Spatial C.V.:	3.3% +/- 1.0% (1.4% to 4.7%)	1.7% +/- 0.5% (1.1% to 2.6%)	4.5% +/- 1.4% (1.9% to 6.6%)
Overall Temporal C.V.:	9.6% +/- 0.7% (8.3% to 10.6%)	8.7% +/- 2.6% (6.1% to 11.7%)	9.7% +/- 4.3% (2.9% to 14.6%)
Mean Concentration, mg/m^3 :	99	105	100

2.4 Particle Size Analysis

The aerodynamic particle size of each test material was determined using a Sierra® Instruments cascade impactor (Model 2210-K, 10 stage). The Sierra impactor was operated at a flow rate of 0.1 CFM for 30 minutes. At this flow rate, particle size could be measured between 18 and .09 µm. The test material was collected on lightly greased stainless steel substrates that were weighed prior to and following sampling to determine mass collected in each size range. Particle size sample data were analyzed by log normal regression, least squares method, of particle size versus cumulative relative mass. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (Øg) of each test material were determined during the calibration and exposure phases of the study. The particle size data are summarized in table 5 and the calculations are contained in Appendix C.

2.5 Animal Observations

Male Fischer 344 rats (CDF/Crl BR) were commercially procured from Charles River Laboratories and were housed in stainless steel suspended cages in racks within the chambers. The Hazelton system is designed and has been tested to hold animals under uniform light, temperature (22° C ± 2° C) and humidity (30-70%) conditions.(6) Certified Laboratory Rodent Chow (#5002) and water was available *ad libitum* . The animals were randomized, weighed and tattooed prior to exposure. Weights were taken at weekly intervals during the pre and post exposure phases and daily during the exposure phase. Prior to initiation of exposures, the animals were examined by the Chief of the Veterinary Services Branch and found to be in good health except for a minor incidence of sialodacryoadenitis which did not jeopardize the outcome of the study. (see appendix D) At the end of the post exposure period, 5 additional control rats were submitted to the US Army Medical Research Institute of Chemical Defense, Veterinary Medical and Laboratory Research Branch for pathogenic quality assurance evaluation. The report is contained in appendix D and no deleterious parasites, pathogens or abnormal histopathology were found.

Just prior to the exposure period, the feed trays were removed from the exposure racks. Following the exposure, the test and control rats were transferred to clean cages in adjacent

TABLE 5. MEAN PARTICLE SIZE OF AEROSOLS

	Natural Graphite MMAD (Øg)	Synthetic Graphite MMAD (Øg)	Titanium Dioxide MMAD (Øg)
During	3.06 (2.19)	2.96 (2.16)	2.15 (3.18)
Calibration	3.04 (2.16)	2.57 (2.26)	2.15 (2.51)
	2.89 (2.22)		
During	2.40 (2.56)	2.34 (2.53)	1.42 (2.40)
Exposure	2.35 (2.66)	2.20 (2.60)	1.59 (2.06)
			1.39 (2.40)
			1.60 (2.10)

MMAD- Mass Median Aerodynamic Diameter in micrometers
Øg- Geometric Standard Deviation

bioclean units while the chambers were cleaned. Toxic signs were observed and recorded before and after each exposure and daily during the pre and post exposure periods.

2.6 Physiological Evaluations

Lung lavage and pulmonary physiological testing were performed on the same animal to enable correlation of biochemical changes with functional changes. At 24 hours and 14 days PE, the rats were anesthetized i.p. with sodium pentobarbital (40 mg/kg) and a tracheal catheter was connected to a Fleish® pneumotachometer for the measurement of respiratory flow. An air-filled esophageal catheter was inserted into the esophagous approximately to the level of the thoracic inlet and was connected to one arm of a Hewlett-Packard® differential pressure transducer for the measurement of esophageal pressure. Transpulmonary pressure (the difference between esophageal pressure and airway pressure derived from a lateral tap at the distal end of the endotracheal tube) was used for all calculations. Both flow and pressure signals were processed in a Buxco Electronics®, Inc. Pulmonary Function Computer and the following parameters were recorded on a Buxco Data Logger: flow, tidal volume, transpulmonary pressure, compliance, and resistance. Compliance, measured by the ratio of the volume change in a tidal breath to the pressure change between end expiration and end inspiration, is a standard physiological method of assessing the overall elasticity or distensibility of the lungs and thorax. Restrictive pulmonary diseases (eg. fibrosis, silicosis) result in decreases in compliance due to a stiffening effect which increases the work of breathing. Resistance is a measure of the pressure difference required for a unit flow change. Inhalation of dusts may lead to an increase in airway resistance. Both compliance and resistance were measured as indicators of functional impairment.

2.7 Bronchoalveolar Lavage

Immediately following the pulmonary measurements, the esophageal catheter was removed and the lavage procedure commenced. The lung washing technique consisted of instilling a calculated volume of normal saline (0.015 ml/g body weight) into the lung and immediately withdrawing the saline until a slight pressure was felt on the syringe plunger. Two lavage washes were done in quick succession. The recovered lavage fluid from both washes was pooled and centrifuged at 300 g for 10 minutes at 4° C.

Following centrifugation, the fluid was separated into supernatant and pellet fractions. The pellet was resuspended in 1 ml 50% bovine serum albumin and total cell counts were taken on a ZBI Coulter Counter®. A differential cell count was made using a modified Pap staining method. The cell pellet was resuspended in Hank's buffered saline; the macrophage concentration was determined in a hemocytometer and cell viability determined via the trypan blue exclusion test.(7) The supernatant lavage fluid was assayed for total protein with the Bio Rad® Protein Assay and for enzymatic activity of lactate dehydrogenase (LDH), alkaline phosphatase (ALKP), and ß-Glucuronidase (ß-Glu). LDH and ALKP were determined on an Abbott VP Series II using an Abbott Analysis Kit and ß-Glu was assayed using a Sigma Chemical Co. kit.

2.8 Pathological Evaluation

At 24 hours and 14 days PE, the test and control rats identified for pathological evaluation were killed using carbon dioxide gas and complete necropsies were performed by Pathology Associates Inc., Ijamsville, Md. in accordance with contract #DAAA15-85-D-0002. All tissues were fixed in 10% neutral buffered formalin, trimmed, dehydrated, embedded in paraffin, sectioned at 6 µm and stained with hematoxylin and eosin. Representative sections were examined for all test groups and controls.

2.9 Data Analysis

Data analysis was conducted according to a statistical "decision tree" as described by Gad and Weil.(8) First, Bartletts's Test for homogenicity of variance was used as a check of the assumption of equivalent variances, followed by the use of ANOVA (analysis of variances). Non-parametric, heterogeneous data was analyzed by the Kruskal-Wallis non-parametric ANOVA. Finally, Dunnett's Test was used on parametric homogeneous data to identify significantly different groups.

3. RESULTS

3.1 Chamber Analyses

The aerosol concentration measurements recorded during each exposure day are tabulated in Appendix E along with the daily statistical computations. These data show that the coefficient of variation for aerosol concentration in any one day was less than 16 percent. The average concentrations for the four days of exposure for each test material were: 102.1 mg/m^3, natural graphite; 100.4 mg/m^3, synthetic graphite; and 101.5 mg/m^3, titanium dioxide. The overall coefficient of variation for concentration was less than 15 percent. To test whether or not the average aerosol concentration was the same for each of the test materials, the Kruskal-Wallis test was applied to the data. A non-parametric test was chosen in place of an analysis of variance since the data failed Bartlett's test for homogeneity of variances at ($p \leq 0.05$). This was because of the greater variance of the titanium dioxide aerosol measurements. The details of the Kruskal-Wallis test are shown in Appendix E. The latter test results indicated that there was no significant difference ($p \leq 0.05$) in the aerosol concentrations of the three materials over the course of the exposure.

Chamber temperature, relative humidity, and flow data are also shown in Appendix E. Note that each value shown represents the average of four measurements. Although relative humidity was measured only in the control chamber for reasons of probe contamination, these values are representative for all four chambers since they all draw air from the same environment. Because of the relatively short residence time (< 2 minutes) of air in the chamber, it is doubtful that the particles would significantly affect the relative humidity of the air. Each chamber contained the same number of animals so that the humidity in the control chamber was representative of all the chambers. Overall these data meet the standards specified in the Guidelines for Good Laboratory Practices.

3.2 Toxic Observations

Throughout the entire study, the control and test animals gained weight at the same rate; there were no statistically significant differences between the groups as depicted in figure 6. The tabulated weight data is contained in Appendix D. During the actual four day exposure period, control and test groups stopped gaining weight but recovered their growth rate during the 14 day post exposure period. This depressive effect on growth was attributed to the stress of exposure. There was concern about the noise generated by the Jet-O-Mizer dispersal system. Before the animals were exposed, the Bio-Acoustics Division of the US Army Environmental

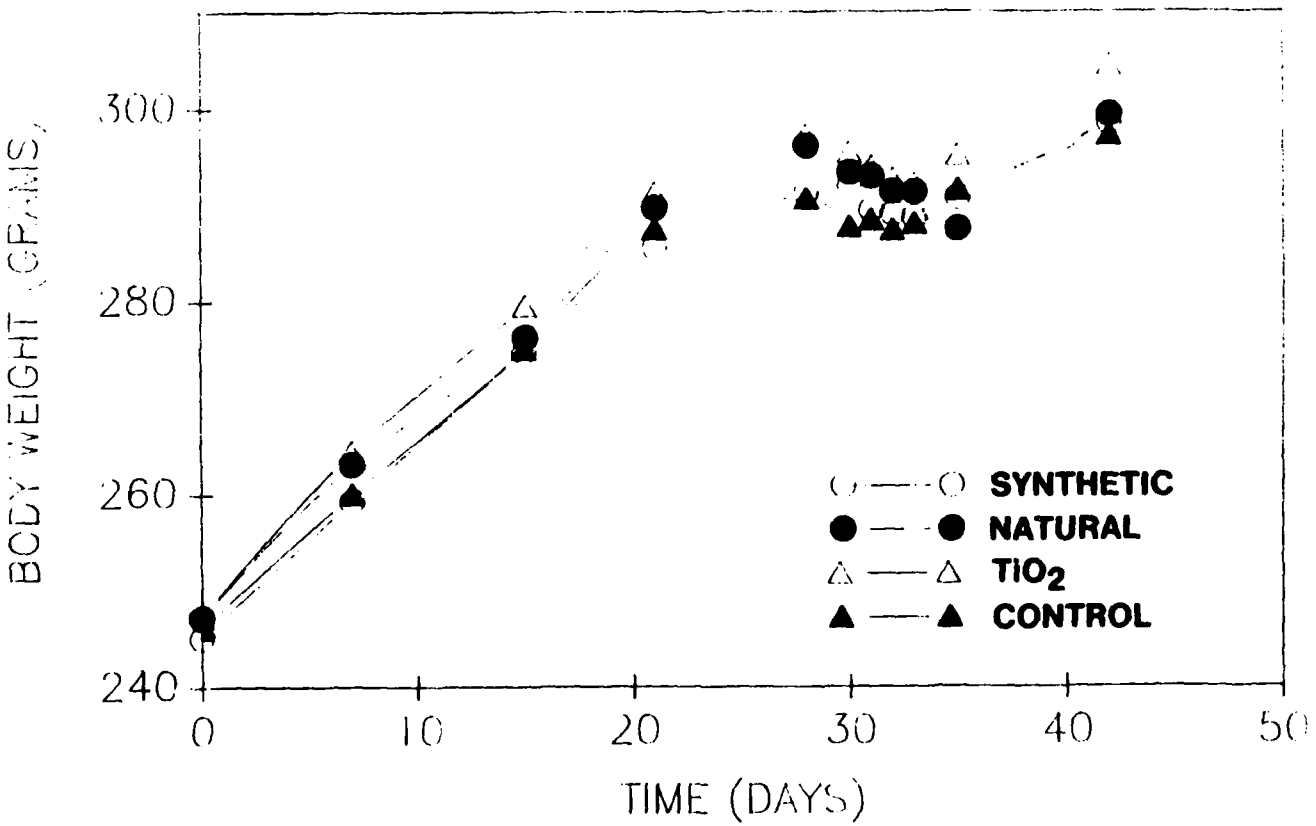


Figure 6. Weights of Test and Control Rats

Hygiene Agency was consulted and measured the noise level in the exposure chambers. Their report is contained in Appendix D, and concluded that the frequency range was outside the region that would adversely affect the rats.

There were no adverse toxic signs exhibited by the animals, normal activity occurred pre and post exposure. The graphite exposed rats were charcoal colored following exposure and remained "dirty " looking throughout the 14 day post exposure period despite some preening.

3.3 Physiological and Bronchoalveolar Response

The results of the pulmonary physiological evaluation of the rats exposed to titanium dioxide and graphite dusts are summarized in tables 6-7. There appears to be a statistically significant decrease in pulmonary resistance at 24 hours PE in the rats exposed to synthetic graphite and a significant increase in respiratory rate at 14 days PE in the rats exposed to titan ium dioxide. Neither of these apparent changes has any biological significance. Previous acute and repeated inhalation studies on synthetic graphite did not result in any consistent significant changes in pulmonary resistance.(1,2)

The enzymatic and protein analyses of the lavage fluid are summarized in table 8. There were significant increases in protein at 24 hours PE with all three dusts but at 14 days all values were within control levels. At 24 hours PE, there were significant increases in ß-Glu and ALKP for both graphite dusts and an increase in LDH for the natural graphite. There was an unexplainable decrease in ALKP for titanium dioxide which may be caused by a material interference with the assay; this effect is being investigated. By 14 days PE, all enzymatic changes were resolved.

Cytological analyses of the lavage fluid are listed in table 9. All three dust exhibited an influx of polymorphonuclear neutrophils (PMN) at 24 hours PE but the graphite dusts elicited a greater PMN response. Likewise, natural graphite exposure resulted in the largest increase in total cells. By 14 days PE, the PMN response had diminished to almost control levels. There was no decrease in macrophage viability from exposure to any of the test materials.

TABLE 6. PHYSIOLOGICAL RESULTS (24 HR PE)

RESPIRATORY PHYSIOLOGY DATA FROM ACUTE INHALATION OF GRAPHITE AND TiO2 EXPOSURE DATES 12/13/87 - 12/16/87 FOUR HOURS PER DAY; FOUR CONSECUTIVE DAYS; 100 mg/m3

GROUP	WEIGHT (Gms.)	FLOW	PLEUR. PRESS.	TIDAL VOL.	COMP-LIANCE	RESIS-TANCE	RESP. RATE	MINUTE VOLUME
CONTROL	293	14.8	6.99	1.82	.312	.169	99.9	181
SD+/-	12	1.8	.68	.13	.067	.028	17.9	37
NATURAL	299	13.5	7.77	1.81	.274	.180	106.1	190
SD+/-	7	2.7	1.16	.17	.043	.066	16.2	18
SYNTHETIC	292	15.4	6.61	1.74	.310	.125	113.2	191
SD+/-	10	2.5	1.92	.21	.090	.036	29.4	28
TiO2	293	12.3	6.08	1.86	.436	.175	98.4	182
SD+/-	6	2.7	1.37	.09	.156	.012	14.2	20
BARTLETT'S TEST	NS	NS	NS	NS	NS	SIG	NS	NS
ANOVA	NS	NS	NS	NS	SIG	NS	NS	NS
DUNNETT'S TEST	-	-	-	C vs NA (NS) C vs SY (NS) C vs Ti (SIG)	-	-	-	-
KRUSKAL-WALLIS NON-PARAMETRIC ANOVA	-	-	-	-	-	SIG	-	-
DISTRIBITION FREE MULTIPLE COMPARISON	-	-	-	-	-	C vs NA (NS) C vs SY (SIG) C vs Ti (NS)	-	-

TABLE 7. PHYSIOLOGICAL RESULTS (14 DAY PE)

GROUP	WEIGHT (Gms.)	FLOW	PLEUR. PRESS.	TIDAL VOL.	COMP-LIANCE	RESIS-TANCE	RESP. RATE	MINUTE VOLUME
CONTROL	308	14.1	6.33	1.81	.343	.136	79.4	145
SD+/-	21	1.4	1.24	.14	.087	.051	15.4	36
NATURAL	310	13.7	7.20	1.74	.269	.171	91.6	160
SD+/-	10	1.6	.92	.09	.028	.049	13.6	27
SYNTHETIC	306	14.1	6.64	1.81	.299	.132	75.9	137
	9	3.3	2.45	.58	.187	.051	11.1	57
TiO2	312	13.6	6.39	1.66	.306	.144	101.9	171
SD+/-	6	1.8	1.50	.12	.068	.043	20.4	32
BARTLETT'S TEST	NS	NS	NS	SIG	NS	NS	NS	NS
ANOVA	NS	NS	NS	NS	NS	NS	SIG	NS
DUNNETT'S TEST	-	-	-	-	-	-	C vs NA (NS) C vs SY (NS) C vs Ti (SIG)	-
KRUSKAL-WALLIS NON-PARAMETRIC ANOVA	-	-	-	NS	-	-	-	-

TABLE 8. Biochemical Results from Analysis of Lavage Fluid

Exposure Material	24 hr P.E.				14 day P.E.			
	B-gluc (Sigma U/mL)	LDH (IU/L)	Alk Phos (IU/L)	Protein (ug/ml)	B-gluc (Sigma U/mL)	LDH (IU/L)	Alk Phos (IU/L)	Protein (ug/ml)
Controls	6.8 ± 0.8	44 ± 13	76 ± 19	400 ± 80	8.3 ± 1.5	47 ± 9	74 ± 10	430 ± 80
Synthetic Graphite	11.8 ± 1.6[b]	58 ± 8	121 ± 10[b]	510 ± 60	7.0 ± 1.5	58 ± 18	80 ± 13	420 ± 50
Natural Graphite	12.6 ± 2.6[b]	72 ± 11[b]	140 ± 14[b]	540 ± 30	7.3 ± 0.5	50 ± 5	74 ± 8	450 ± 120
TiO_2	7.4 ± 1.2	51 ± 14	39 ± 5[b]	540 ± 90	7.3 ± 2.3	59 ± 6	58 ± 8	480 ± 160

[a] Significantly different from controls by Dunnett's test ($P \leq 0.05$)

[b] Significantly different from controls by Dunnett's test ($P \leq 0.01$)

Table 9 CYTOLOGICAL ANALYSIS OF BRONCHOALVEOLAR LAVAGE FLUID

24 Hour Post Exposure

		WBC $x10^3$	TOTAL x104	VIABILIITY %	MACROPHAGE %	LYMPH %	PMNS %
CONTROL	x	2.22	4.34	97	98	2	0
	s	0.50	1.51		2	1	0
SYNTHETIC	x	2.40	5.18	95	56	1	43
	s	0.46	0.89		7	0	7
NATURAL	x	3.37*	6.10	94	46	2	52
	s	0.73	1.26		10	1	10
TITANIUM	x	1.45	6.22	96	85	4	11
OXIDE	s	1.20	2.07		15	5	11

14 DAY POST EXPOSURE

CONTROL	x	2.00	4.41	98	97	2	1
	s	0.66	1.11		2	1	1
SYNTHETIC	x	2.13	5.29	98	92	3	5
	s	0.43	1.01		7	3	4
NATURAL	x	2.58	6.12*	99	92	2	6
	s	0.57	0.61		4	1	4
TITANIUM	x	1.88	3.67	98	94	2	4
OXIDE	s	0.42	0.53		3	2	3

* significant p=0.05 (t-test)

3.4 Pathological Evaluations

The complete pathology report from Pathology Associates Inc. is contained in Appendix F. The gross observations noted at the time of necropsy indicated that several of the graphite exposed rats had discolored or mottled lungs. There were no apparent differences in body or organ weights. The organ/body weight ratios are summarized in Appendix D.

Treatment related changes were present in the lungs of all exposed rats consisting of brown to black, isotropic pigment. At 24 hours PE in all cases, the pigment was present either free or within macrophages in terminal airways and alveoli. Microscopically, the three types of pigment were indistinguishable from each other. There was no pigment in the peribronchial lymph nodes and no adverse tissue reaction to it. By 14 days PE, there was no free pigment (extracellular) in the lungs of the exposed rats. Again, the three types of pigment were indistinguishable; however, in the graphite exposed rats, the pigment-laden macrophages tended to be aggregated in small groups more than in the titanium dioxide exposed rats. The only other changes were two minimal foci of epithelial hyperplasia in the alveoli and/or the terminal bronchioles of three rats exposed to synthetic graphite and one rat exposed to titanium dioxide. The pigmented macrophages were not associated with the hyperplasia. It was concluded that the degree of pigmentation was mild in all exposed rats and nearly identical within and between groups.

All tissues and original data are stored in the archives of CRDEC, Research Directorate, Toxicology Division.

4. DISCUSSION

Inhalation exposure of Fischer 344 male rats to 100 mg/m^3 of titanium dioxide, natural and synthetic graphite dusts for 4 hrs/day for four days resulted in minimal adverse effects. There were no adverse toxic signs following exposure, no mortality and no consistent pulmonary function changes. All the rats gained weight at the same rate as the controls. BAL analyses resulted in increases in protein for all three materials; increases in ß-Glu and ALKP for both graphites; and increases in LDH for natural graphite at 24 hours PE. The increase in LDH is reported to be indicative of damage to the pulmonary Type I cells while ALKP increases may be correlated with Type II cell hyperplasia.(9) Type II cell hyperplasia was only observed in three rats at 14 day PE, two from the synthetic graphite exposed group and one from the titanium dioxide exposed group. This effect was observed in a previous study where rats were exposed to 100 mg/m^3, 2 hours/day, 5 days/wk for two weeks.(2) Perhaps a longer repeated exposure would have resulted in more Type II cell hyperplasia in this study. Since Type II cells

are the progenitors of Type I cells (10), this would be an indicator that the damaged alveolar epithelium is undergoing repair and replacement. The graphite exposed rats also had increases in ß-Glu which is a lysosomal enzyme released by phagocytic cells in response to inflammation.(11) These enzymatic changes correlate with the BAL cytological profile (i.e. increases in PMN and total nucleated cells), which are indicative of an inflammatory response. By 14 days PE, all BAL alterations were resolved.

The histopathological evaluation revealed mild lung pigmentation in all the exposed rats with more aggregates of pigment in the graphite exposed rats at 14 days PE. The macrophages seen in the alveoli appear to be actively phagocytizing all three materials. There was no decrement in macrophage viability which is in agreement with previous studies with synthetic graphite and titanium dioxide.(12) No pigment was observed in the peribronchial lymph nodes; this was expected since in prior inhalation studies with graphite pigmentation in the lymph nodes was not evident until 3 months PE. Clearance of these dusts may be a slow, protracted process.

The BAL changes seen after repeated exposure to graphite were more severe than the changes following a single exposure. Previous acute inhalation exposure to graphite resulted in minimal cytological changes reversible by 14 days PE and no enzymatic BAL changes.(1) However, the BAL response in this study is mild compared to the dramatic inflammatory reactions observed in acute studies with brass powder(13), and is not as persistent as the effects seen with aluminum(13) and quartz.(14) In the latter study following a single 100 mg/m^3 inhalation exposure to quartz, BAL enzymes were elevated two to five hundred percent over controls at 3 days and 3 months PE. The changes with graphite were minimal and reversible.

The repeated inhalation studies in this report and the previous acute inhalation exposure to synthetic graphite even at very high concentrations (500 mg/m^3) does not result in any permanent effects. This is in agreement with the OSHA (15) and Documentation of TLVs (3) guidelines which regard synthetic graphite as a nuisance dust. The higher quartz content (>1%) of natural graphite supposedly accounts for the greater risk of developing fibrosis; thus, natural graphite is assigned a TLV of 2.5 mg/m^3. However, this hypothesis is not conclusive; a survey of the literature on the etiology of coal-workers' pneumoconiosis (CWP) reveals uncertainty as to what part quartz plays in pathogenesis.(16) Several studies in animals have implicated quartz as the causative factor in mixed dust fibrogenesis. Martin et al. (17) found collagen formation after 18 months in the lungs of rats that had inhaled a coal mixture with 5%

quartz for 90 days. At concentrations above 10% quartz, the formation of fibrotic nodules and collagen occurred at a rate five times higher than coal alone. Further confirmation of this theory was demonstrated by Schlipkoter et al. (18) in experiments where quartz, coal and titanium dioxide, alone and in mixtures, were administered to rats intraperitoneally. Fibrosis was induced when quartz was added to the mixtures and the authors concluded that whenever quartz is present in a particular mine dust producing CWP , it should be considered the dangerous agent. This interpretation according to Parkes (16) is contradicted by a number of observations in human beings. Both simple pneumoconiosis (benign dusty lung) and progressive massive fibrosis have occurred in men exposed to artificial or quartz-free graphite.(19-21) In each case, quartz was absent or less than 1% in the lungs; therefore, such instances imply quartz is not the pathogenic factor. The controversy is more than an academic debate since occupational exposure standards are based upon the quartz content of the dust in question (eg. graphite). Recent epidemiological studies in British mines showed that an apparent increase in the prevalence of pneumoconiosis with increasing quartz exposure is reversed in the presence of high clay mineral exposure (aluminum silicate clays are known to inhibit silicosis) and that mass concentration of respirable dust is the best exposure index when the quartz content does not exceed 7.5%. This "mass" effect of dust exposure has been recently demonstrated by the results of chronic inhalation studies conducted with titanium dioxide. Lee et al. (22) found fibrosis and bronchoalveolar adenomas in the lungs of rats exposed to 250 mg/m^3 of titanium dioxide for 6 hrs/day, 5 days/wk, for 2 years. The pulmonary lesions were the result of overwhelming the lung clearance mechanisms.

5. CONCLUSIONS

Repeated inhalation exposure of Fischer 344 rats to 100 mg/m^3 of titanium dioxide, natural graphite, and synthetic graphite for 4 hours/day for four days resulted in a mild inflammatory response 24 hours PE. BAL changes were the most sensitive indicator of damage;although the enzymatic and cytological alterations were evident with all three materials, there were greater increases with the graphite dusts. Even though the graphite dusts and titanium dioxide were still present in the alveolar macrophages of each respective group of rats, by 14 days PE, all BAL changes were resolved. This seems to indicate that the initial period of inflammation had ceased and a slow clearance was in process. There appears to be no deleterious tissue reaction to any of the materials at the levels tested in this study.

In this experiment, synthetic graphite, natural graphite, and titanium dioxide meet the criteria of the ACIGH for a nuisance dust: (1) the architecture of the air spaces remained intact; (2) collagen (scar tissue) was not formed; and (3) the tissue reaction was potentially reversible. Repeated exposure to graphite dust results in more pulmonary damage than single exposures. If the nuisance dust TLV (10 mg/m^3) is exceeded, respiratory protection should be utilized.

Additional studies should be conducted to investigate the effects of subchronic inhalation exposure to graphite dust. In these studies, a satellite group of test animals should be held 6 months PE to ascertain the length of time required for dust clearance.

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APPENDIX A. STUDY PROTOCOL AND AMENDMENTS

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DISPOSITION FORM

For use of this form, see AR 340-15 the proponent agency is TAGO

REFERENCE OR OFFICE SYMBOL	SUBJECT
SMCCR-RST-E (70-1p)	Amendment to Protocol Entitled, "Comparative Acute Inhalation Screen of Iron Oxide and Graphite Dust"

TO HVSO
ATTN: Chairman, LAURC

FROM Chief, Environ Tox Br

DATE 7 Dec 87

CMT 1

Dr. Thomson/rlp/3762

1. Request amendment to subject protocol to substitute the use of titanium dioxide (TiO_2) for iron oxide.

2. Due to technical difficulties, iron oxide dust could not be generated at the concentration for the duration required. TiO_2 is a nuisance dust that meets the requirements of the sponsor, US Army Biomedical Research and Development Laboratory.

3. Enclosed is a copy of the material safety data sheet.

4. The principal investigator is Dr. Sandra Thomson, x3762.

Encl
as

JOHN T. WEIMER
Chief, Environmental Toxicology Branch

SMCCR-HV (15-1a)

TO SEE DISTRIBUTION FROM CDR, CRDEC DATE 9 Dec CMT 2

The Amendment to Protocol Entitled "Comparative Inhalation Screen of Iron Oxide and Graphite Dusts, 87PP7870," has been reviewed and administratively approved by the Chairman, LAURC.

FOR THE COMMANDER

Encl
nc

CARL W. JOHNSON
LTC, MS
Chief, Hlth & Vet Svcs Ofc

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NL Chemicals, Inc.
P O Box 700 Hightstown N J 08520 U S A
Te: 609) 443-2500 Telex 642240

NL Chem Canada, Inc.
[illegible] Place Ville-Marie Suite 500 Montreal PQ H3B 4M5
Te: 514 397-3501 Telex 0526866

NL Chemicals Europe, Inc.
[illegible] Bte 6 B 1000 Brussels Belgium
Te: (02) 512 0048 Telex 24662 NL EUR B

[redacted]

DESCRIPTION

TITANOX 3020 pigment is a free flowing, coarse particle size, high purity grade of titanium dioxide designed specifically for the various glass manufacturing processes.

TYPICAL PHYSICAL PROPERTIES

Crystal Structure	Principally Rutile
Bulk Density, pounds per cubic foot	75
Moisture	0.7% maximum
Typical Composition	
TiO_2	99.5%
Iron	50 ppm
Chromium	5 ppm
Vanadium	15 ppm
Typical Particle Size	
+35 Mesh	35%
+325 Mesh	75-85%

APPLICATIONS

■ CONTAINER AND FLAT GLASS: As a component of the glass batch, **TITANOX 3020** pigment often lowers the melting point concurrently reducing the high temperature viscosity which can result in operating economies. In the finished glassware, the use of **TITANOX 3020** pigment results in decreased transmission in the ultraviolet range - or, in colored glasses, shifts the transmission in the visible region to longer wave-lengths. **TITANOX 3020** pigment improves the chemical durability of glass - especially in acidic environments. The brilliance of glass is improved by an increase in the refractive index and dispersion of the glass.

■ GLASS BEADS: Owing to its excellent handling qualities, chemical purity and high index of refraction, **TITANOX 3020** pigment is recommended for the manufacture of reflective glass beads.

■ OPTICAL GLASS: **TITANOX 3020** pigment finds application in optical or specialty glasses where purity of the raw materials is essential. Refractive index and dispersion of the glass can be controlled by use of **TITANOX 3020** pigment.

NL Chemicals MATERIAL SAFETY DATA SHEET

Day (609) 443-2461
Emerg # 24 hrs. (609) 443-2000
CHEMTREC (800) 424-9300

SECTION I

MANUFACTURER'S NAME: NL CHEMICALS / NL INDUSTRIES, INC. · ENVIRONMENTAL CONTROL DEPARTMENT
DATE: 8/7/85
STREET ADDRESS: P.O. BOX 1090, WYCOFF HILLS RD., HIGHTSTOWN, N.J. 08520
REASON FOR ISSUE: Update 10/17/84
TSCA INFO: ☐ MIXTURE*
PRODUCT CLASS: Pigment-Titanium Dioxide
MANUFACTURER'S PRODUCT NO.: All Grades
TRADE NAME: TITANOX®
☐ CHEM SUB CAS 13463-67-7

SECTION II - HAZARDOUS INGREDIENTS

INGREDIENT	PERCENT BY WEIGHT	TLV PPM	TLV mg/m³	LEL %	VAPOR PRESSURE mm Hg @ 68°F
Titanium Dioxide	80% Min.		15.0 (1) 10.0 (2)	-	-

(1) OSHA Standard, 29 CFR 1910.1000 Table Z-1
(2) As a nuisance dust - ACGIH

SECTION III - PHYSICAL DATA

BOILING RANGE: N/A

VAPOR DENSITY	EVAPORATION RATE	% VOLATILE BY VOLUME	WEIGHT PER GALLON	SPECIFIC GRAVITY
☐ HEAVIER N/A ☐ LIGHTER THAN AIR	☐ FASTER N/A ☐ SLOWER THAN ETHER	N/A	N/A	3.7 - 4.2

SECTION IV - FIRE AND EXPLOSION HAZARD DATA

DOT CATEGORY: Not Hazardous
FLASH POINT: N/A
LEL: N/A

EXTINGUISHING MEDIA
☐ WATER-FOG ☐ FOAM ☐ OTHER
☐ CARBON DIOXIDE ☐ DRY CHEMICAL N/A

UNUSUAL FIRE AND EXPLOSION HAZARDS
None.

SPECIAL FIRE FIGHTING PROCEDURES
None.

SECTION V - REACTIVITY DATA

STABILITY: ☐ UNSTABLE X ☐ STABLE
CONDITIONS TO AVOID

INCOMPATABILITY (MATERIALS TO AVOID)
None.

HAZARDOUS DECOMPOSITION PRODUCTS
None.

HAZARDOUS POLYMERIZATION: ☐ MAY OCCUR X ☐ WILL NOT OCCUR
CONDITIONS TO AVOID: None.

N/A—NOT APPLICABLE
* COMPONENTS IN TSCA INVENTORY

SECTION VI - HEALTH HAZARD DATA

15.0 mg/m³ based on OSHA Standard (as a nuisance dust).

EFFECTS OF OVEREXPOSURE

Irritating to respiratory system.

EMERGENCY AND FIRST AID PROCEDURES

Eyes - Normal washing to remove dust.

Inhalation - Remove to fresh air.

Ingestion - Call a physician.

SECTION VII - SPILL OR LEAK PROCEDURES

STEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED

Clean area returning all material possible to container.

WASTE DISPOSAL METHOD

Dispose in accordance with federal, state or local regulations.

SECTION VIII - SPECIAL PROTECTION INFORMATION

RESPIRATORY PROTECTION

NIOSH approved respiratory protection must be used in accordance with existing standards.
Use NIOSH approved respirator if TLV is exceeded.

VENTILATION

Provide as required to keep TLV below acceptable limits.

PROTECTIVE GLOVES Yes.
EYE PROTECTION Yes. Goggles.
OTHER PROTECTIVE EQUIPMENT None special.

SECTION IX - SPECIAL PRECAUTIONS

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORING

Avoid breathing dust.
Use with adequate ventillation.

OTHER PRECAUTIONS

NPCA Hazardous Materials Identification System (HMIS):

None.

Health 1; Flammability 0; Reactivity 0; Personal Protection

N/A—NOT APPLICABLE

RESEARCH PROTOCOL

TITLE: Comparative Acute Inhalation Screen of Iron Oxide and Graphite Dusts.

DIRECTOR/DIVISION: Toxicology Division, Research Directorate, Chemical Research Development and Engineering Center, Aberdeen Proving Groiund, MD 21010-5423.

RESPONSIBLE INVESTIGATOR(S):

Principal Investigator:
Sandra Thomson, Ph.D.

Co-Investigators:
Dave Burnett

John Carpin, Ph.D.

Quality Assurance:
Dennis Johnson

Branch Chief:
John T. Weimer
Chief, Environ Tox Br

Division Chief:
Harry Salem, Ph.D.
Chief, Toxicology Division

Director:
for Edward J. Poziomek, Ph.D.
Director, Research

SPONSOR: U.S. Army Biomedical Research and Development Laboratory, Fort Detrick, MD

Dr. Dickinson Burrows 15 Jul 87
Date

MANAGEMENT DATA:

Project No.
Protocol No. 22087030A217

Job Order No.
Start Date: July 1987
Completion Date: September 1987

1. BACKGROUND:

Synthetic and/or natural graphite dust may have military applications which could result in inhalation hazards. CRDEC has tested synthetic (Asbury Micro 260) and natural (Asbury Micro 650) graphites and found that acute inhalation exposure resulted in mild reversible inflammatory response at high concentrations (500 mg/m^3) for the synthetic material. A repeated inhalation study with the synthetic graphite also showed more changes at a lower concentration (100 mg/m^3) reversible at 3 months post-exposure (PE). The acute inhalation study for the natural graphite is still in process but initial results at 24 hrs PE showed greater bronchoalveolar lavage (BAL) changes compared to the synthetic material. A repeated (14 day) comparison study of the natural graphite is planned to better define the differences between the natural vs. synthetic graphite materials.

USAMBRDL, the sponsor for this study has suggested an additional experiment to differentiate the physiologic vs pathologic (toxic) response to dust loading of the lung. In this experiment, the effects of natural and synthetic graphite will be compared to iron oxide dust. Epidemiologic and animal studies have shown iron oxide to be noncarcinogenic[1]. The pulmonary effect of iron oxide is siderosis (iron pigmentation) without physical disability; however, the time weighted average TLV has been reduced to 5 mg/m^3 (total particulate as Fe) to prevent development of x-ray changes in the lung on long-term exposure[2]. The short-term exposure limit (STEL) has been eliminated until additional toxicological data and industrial hygiene experience becomes available to better define the basis for a STEL.[2]

The synthetic graphite used in this study is Asbury Micro 260 (less than 1% silica) and the natural graphite is Asbury Micro 650 (1.85% silica); both contain neglible amounts of contaminants (encl 1). The iron oxide selected is iron oxide (R-2999RP, 99.6% pure), a gift from Pfizer, Inc. Minerals, Pigments and Metal Division, Easton, PA (encl 2). This iron oxide is the same material used by Beck et. al. in their intratracheal instillation studies where they showed that toxicity indicators for iron oxide approached control levels four days following exposure.[3] This suggests pure iron oxide acts as a "nuisance dust" producing reversible tissue reaction, no increases in collagen nor changes in the architecture of the air spaces as defined by ACGIH.[4]

2. HYPOTHESIS:

Airborne exposure of rats to equal concentrations of natural, synthetic graphites and iron oxide will produce no significant histopathological differences in the lungs of exposed animals.

3. MATERIALS AND METHODS:

Experimental Design:

Groups of 20 male Fischer 344 rats will be exposed by inhalation to 100 mg/m^3 of natural graphite, synthetic graphite and iron oxide on four consecutive days, four hours/day. Fischer 344 rats are the species of choice because they were used in previous studies with graphite. Microscopic evaluation of the respiratory tract and major organs will be done at two time periods post-exposure (PE) in the following manner:

Material	# of Rats	
	1 day PE	14 days PE
Micro 260 (Synthetic)	10	10
Micro 650 (Natural)	10	10
Iron Oxide	10	10
Control	10	10

Species: Rat
Breed: Fischer 344
Total # 85 (5 additional rats are required for quality assurance).
Sex and Age: Male, 12 weeks, 175-200g

4. CHAMBER OPERATION AND SAMPLE COLLECTION:

The rats will be exposed in four 1000 liter Hazelton chambers located in Bldg E3266, Room 3.

The dusts will be delivered to the intake of the chamber by a Jet-O-Mizer aerosol generator. The concentration in the chamber is maintained by varying feed and blower speeds of the generator and chamber air flow and is monitored via gravimetric methods. Sampling is conducted at a rate of 5 L/minute for 10 minutes for each sample 6-12 times during each exposure. Prior to the start of exposures, calibration of chambers will be conducted to assure a stable concentration. Pre-filtered room air is the air source and the temperature and humidity of the chamber is maintained at 22°C $\pm$ 2° and 30 - 70%, respectively (in accordance with OECD guidelines). The air flow is maintained to insure chamber oxygen content of at least 19%. The exhausted air is filtered through another particulate filter.

The Mass Median Aerodynamic Diameter (MMAD) will also be measured for each concentration using a Sierra Cascade impactor. The particle size is expected to be within a respirable range.

5. TECHNICAL METHODS:

5a. Animal Holding

The rats will be housed in stainless steel suspended cages in racks within the chambers. The Hazelton system is designed and has been tested to hold animals under uniform light, temperature (22°C $\pm$ 2°C) and humidity (30 - 70%) conditions (Beethe et. al., 1979).[5] Two weeks prior to the start of the

experimental period the animals will be acclimated to the chambers. Certified Commercial rodent diet and water will be available ad lib. Cage fecal trays will be changed daily and exposure chambers will be cleaned immediately following each day's exposure. During this period the test animals will be transferred to clean cages in Bioclean laminar flow units.

5b. Handling During Testing

Animals will be randomized, weighed and tattooed prior to exposure. An on-call veterinarian will be consulted if the animals appear to suffer undue distress or disease processes during the course of the experiment. Toxic signs will be observed and recorded before and after the exposure period. Animals will be weighed at weekly intervals before and during the experimental phase and post-exposure periods.

At the end of the experimental period, all scheduled rats will be submitted and euthanatized with carbon dioxide, necropsied and their tissues prepared for light microscopic examination by Path. Assoc. Inc. in accordance with contract #DAAA15-85-0002. During necropsies, the animal total body weight and the following organ weights will be recorded: heart, lung, liver, kidneys and gonads.

5c. USDA Pain Category: No Pain

6. DATA ANALYSIS PLAN:

Data analysis will be conducted according to a statistical "decision tree" as described by Gad and Weil (1984).[6] First, Bartletts's Test for homogenicity of variance will be used as a check of the assumption of equivalent variances, followed by the use of ANOVA (analysis of variances). Non-parametric, heterogeneous data will be analyzed by the Kruskal-Wallis non-parametric ANOVA. Finally Duncan's Multiple Range Test will be used on parametric homogeneous data to identify significantly different groups.

7. COORDINATION:

a. Pathology. Prior to the start of the experiment, there will be coordination with USAMRICD, Veterinary Medicine & Surgery Branch, for animal procurement, identification, and health certification; and with Pathology Associates Inc. for gross necropsies and preparation of slides for histopathology. Slides will be prepared and read by Pathology Associates Inc., our designated contractor. Necropsies of spontaneous deaths will be coordinated with USAMRICD, Comparative Pathology Branch.

b. Animal Requirements

Species: Fischer 344 Rats
Sex, Weight, Age: Male, 12 wks. old, 175-200g
Total number: 85
Starting date: July 1987

c. Cost Accounting

Job Order #:
Project #:

8. This study will be consistent with Good Laboratory Practice. Maintenance and use of animals will be in accordance with the guidelines contained in NIH publication 85-23 (Guide for the Care and Use of Laboratory Animals).

a. Control of Bias: Every reasonable attempt will be made to control bias throughout the experiment. Animals will be rotated within the chamber on a scheduled pattern. Since there will be dust deposition on the exposed animals, it will be obvious to the technicians recording toxic observations which animals are exposed.

b. Record Maintenance: All chamber analysis data, toxic obervations and animal weights will be recorded in official CRDEC notebooks. All other associated raw data (statistical printouts, necropsy incidence tables, etc.) will be stored in the CRDEC, Research Dir, Toxicology Div Archives.

REFERENCES

1. Stokinger, H.E., A Review of World Literature Final Iron Oxides Noncarcinogenic", Am. Incl. Hyg. Assoc J., 45 (2): 127-133 (1984).

2. Documentation of the Threshold Limit Values and Biological Exposure Indices, 5th ed. Amer. Conf. Govt. Indus. Hyg., Cincinnati, Ohio (1986).

3. Beck, Barbara, Brain, J., Bohannon, D., An in Vivo Hamster Bioassay to Assess the Toxicity of particulates for the Lungs Tox. Appl. Phar., 66: 9-29 (1982).

4. Amer. Conf. Govt. Indus. Hyg., Threshold Limit Value and Biological Exposure Indices for 1986-1987, p. 6.

5. Beethe, R.L., Wolff, R.K., Griffis, L.C., Hobbs, C.H., McClellan, R.O, 1979, Evaluation of a Recently Designed Multi-Tiered Exposure Chamber. Rsch. Inst. Report LF-67.

6. Gad, S., Weil, C., Statistics for Toxicologists in Principles and Methods of Toxicology, ed. Hayes, Raven Press, NY, 273-320 (1984).

LABORATORY
OF
Asbury Graphite Mills, Inc.

Asbury, Warren County, N. J. • Telephone: (201) 537-2155

Analysis No.

Grade No. Micro #2 60

Sample Taken From

TYPICAL CHEMICAL ANALYSIS

Carbon	99.9%
Ash	0.1
Sulfur	Not Detectable

CHEMICAL ANALYSIS OF ASH

Si	0.008
Al	0.0009
Fe	0.009
Ca	0.011
Ti	0.003
Mg	0.0001

TYPICAL PHYSICAL ANALYSIS

Carbon	99%
Scott Vol. Wt/cu/in.	2.67 grams
Fisher Sub-Sieve Sizer A.P.D.	.61 microns

These percentages are not guaranteed, they are included only to indicate the approximate physical and chemical analysis.

Asbury Graphite Mills, Inc.
C. Brochini
By

Date

REPORT OF ANALYSIS

LABORATORY

OF

Asbury Graphite Mills, Inc.

Asbury, Warren County, N. J. • Telephone: (201) 537-2155

Analysis No.

Grade No. Micro 650

Sample Taken From

MICRO #650

TYPICAL ANALYSIS

Fineness	.54 Microns A.P.D.
-325 Mesh	100%
Carbon	96.60%
Ash	3.40%

SIZE AND CARBON DATA

Fineness .50 to .60 Microns A.P.D. as determined by a Fisher Sub-Sieve Sizer.

+325 Mesh	None
-325 Mesh	100%
Carbon	95% Min.
Ash	5% Max.

GENERAL CHEMICAL ANALYSIS

Moisture	.05%
Volatile	.10%
Carbon	96.20%
Ash	3.65%

COMPOSITION OF ASH

Silica	1.85%
Alumina	.95%
Iron Oxide	.30%
Calcium Oxide	.20%
Manganese Oxide	.05%
Sulphuric Trioxide	.10%
Potassium Oxide	.10%

These percentages are not guaranteed, they are included only to indicate the approximate physical and chemical analysis.

Asbury Graphite Mills, Inc.

By LCZ

Date

DISPOSITION FORM

For use of this form, see AR 340-15, the proponent agency is TAGO

REFERENCE OR OFFICE SYMBOL	SUBJECT
SMCCR-RST-E	Addendum to Protocol Entitled, "Comparative Acute Inhalation Screen of Iron Oxide and Graphite Dust"

TO HVSO
ATTN: Chairman, LAURC

FROM Chief, Environ Tox Br

DATE 29 June 87

CMT 1
Dr. Thomson/rlp/3762

1. Request addendum to subject protocol to include additional animals for bronchoalveolar lavage studies.

2. Rationale for request is as follows:

a. Additional rats are requested under F7J4 funding for bronchoalveolar lavage (BAL), since the subject protocol is funded by USABRDL and does not include BAL studies.

b. In previous studies, BAL has proven to be the most sensitive indicator of pulmonary damage, and these additional studies would enhance the CRDEC, Tox Div data base at minimal cost.

3. The methods of analysis are previously described in Protocol #21087000A207.

4. The animal data requirement is as follows:

Material	# of Rats	
	1 day PE	14 days PE
Micro 260	6	6
Micro 650	6	6
Iron Oxide	6	6
Control	6	6
	24	24

Species: Rat
Breed: Fischer 344
Total #: 48
Sex and Age: Male, 12 wks, 175-200g

5. The principal investigator is Dr. Sandra Thomson, ext 3762.

JOHN T. WEIMER
Chief, Environmental Toxicology Branch

DA FORM AUG 80 2496

APPENDIX B. STATISTICS FOR CHAMBER CALIBRATION

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One-Way Analysis of Variance

Aerosol Concentration (mg/m^3): Chamber #2 - Natural Graphite
Null Hypothesis: There is no difference between sampler locations

Location :	Southwest	Center	Southeast	Northeast
N:	14	14	14	14
Minimum:	89.2	85.6	87.6	87
Mean:	102.7	98.29286	102.05	98.47857
Maximum:	118	116	119	110
Sum:	1437.8	1376.1	1428.7	1378.7
Std. Dev.:	10.13744	9.541284	10.24843	9.312454
Std. Err.:	2.709345	2.550016	2.73901	2.488858
95% C.L.:	5.852186	5.508034	5.916261	5.375934

Location :	Northwest
N:	14
Minimum:	80.9
Mean:	95.55714
Maximum:	110
Sum:	1337.8
Std. Dev.:	9.56972
Std. Err.:	2.557615
95% C.L.:	5.52445

Source of Variation	Sum of Squares	Deg. of Freedom	Mean Square	F Value
Total:	6689.387	69		
Between:	486.625	4	121.6563	1.27
Within:	6202.762	65	95.4271	

F (95%): 2.52 F (99%): 3.65

Differences between means: NOT Significant at $p < 0.05$

One-Way Analysis of Variance

Aerosol Concentration (mg/m^3): Chamber #3 - Synthetic Graphite
Null Hypothesis: There is no difference between sampler locations

Location :	Southwest	Center	Southeast	Northeast
N:	13	13	13	13
Minimum:	90.4	90.6	87.5	89.3
Mean:	106.5846	105.0462	104.2769	105.0769
Maximum:	123	124	122	120
Sum:	1385.6	1365.6	1355.6	1366
Std. Dev.:	9.261196	9.770075	9.359056	8.911524
Std. Err.:	2.568594	2.709731	2.595735	2.471612
95% C.L.:	5.596966	5.904504	5.656107	5.385642

Location :	Northwest
N:	13
Minimum:	89.3
Mean:	104.0231
Maximum:	123
Sum:	1352.3
Std. Dev.:	9.817768
Std. Err.:	2.722959
95% C.L.:	5.933328

Source of Variation	Sum of Squares	Deg. of Freedom	Mean Square	F Value
Total:	5387.376	64		
Between:	51.9375	4	12.98438	.15
Within:	5335.439	60	88.92398	

F (95%): 2.52 F (99%): 3.65

Differences between means: NOT Significant at $p < 0.05$

One-Way Analysis of Variance

Aerosol Concentration (mg/m^3): Chamber #4 - Titanium Dioxide
Null Hypothesis: There is no difference between sampler locations

Location :	Southwest	Center	Southeast	Northeast
N:	10	11	11	11
Minimum:	77.8	75.7	73.4	80.4
Mean:	101	100.9636	94.15455	101.0182
Maximum:	119	115	114	117
Sum:	1010	1110.6	1035.7	1111.2
Std. Dev.:	11.69834	12.85568	12.13354	10.44613
Std. Err.:	3.699339	3.876134	3.658399	3.149627
95% C.L.:	8.367905	8.636028	8.150913	7.017369

Location :	Northwest
N:	11
Minimum:	80
Mean:	101.3727
Maximum:	114
Sum:	1115.1
Std. Dev.:	10.30612
Std. Err.:	3.107413
95% C.L.:	6.923318

Source of Variation	Sum of Squares	Deg. of Freedom	Mean Square	F Value
Total:	6932.514	53		
Between:	422.5625	4	105.6406	.8
Within:	6509.951	49	132.8561	

F (95%): 2.61 F (99%): 3.83

Differences between means: NOT Significant at $p < 0.05$

Blank

APPENDIX C. PARTICLE SIZE DATA

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Particle Size Sample Data of Natural Graphite Taken During Calibration

Stage	Dp50	Tare Wt. mg	Sample Wt.	mg	Cumul. Tot.	Cumul. %
F	---	94.90	95.03	0.13	0.13	0.97
10	----------	----------	not used	----------	----------	----------
9	.16	308.01	----	----	0.13	0.97
8	.32	313.94	----	----	0.13	0.97
7	.53	310.78	311.06	0.28	0.41	3.06
6	.95	314.78	316.18	1.40	1.81	13.49
5	1.70	357.39	361.16	3.77	5.58	41.58
4	2.65	308.06	313.33	5.27	10.85	80.85
3	4.40	313.00	315.52	2.52	13.37	99.63
2	11.0	354.19	354.24	0.05	13.42	100
1	18.0	303.70	------	----	-----	-----

Stage	Dp50	Tare Wt. mg	Sample Wt.	mg	Cumul. Tot.	Cumul. %
F	---	102.59	102.66	0.07	0.07	0.45
10	----------	----------	not used	----------	----------	----------
9	.16	304.90	------	----	0.07	0.45
8	.32	312.35	------	----	0.07	0.45
7	.53	313.50	313.63	0.13	0.20	1.29
6	.95	309.52	311.50	1.98	2.18	14.02
5	1.70	357.96	362.38	4.42	6.60	42.44
4	2.65	308.56	314.76	6.20	12.80	82.32
3	4.40	355.67	358.42	2.75	15.55	100
2	11.0	314.76	------	----	-----	-----
1	18.0	312.11	------	----	-----	-----

Particle Size Sample Data of Natural Graphite Taken During Calibration

Stage	Dp50	Tare Wt. mg	Sample Wt.	mg	Cumul. Tot.	Cumul. %
F	---	97.60	97.70	0.10	0.10	0.61
10	----------	----------	not used	----------	----------	----------
9	.16	354.94	------	----	0.10	0.61
8	.32	358.64	------	----	0.10	0.61
7	.53	309.71	310.15	0.44	0.54	3.30
6	.95	314.04	316.62	2.58	3.12	19.09
5	1.70	313.44	317.83	4.39	7.51	45.96
4	2.65	309.66	315.64	5.98	13.49	82.56
3	4.40	314.34	317.19	2.85	16.34	100
2	11.0	314.05	------	----	-----	-----
1	18.0	304.36	------	----	-----	-----

Particle Size Sample Data of Natural Graphite Taken During Exposure

Stage	Dp50	Tare Wt. mg	Sample Wt.	mg	Cumul. Tot.	Cumul. %
F	---	118.08	118.20	0.12	0.12	1.05
10	----------	----------	not used	----------	----------	----------
9	.16	308.04	308.18	0.14	0.26	2.28
8	.32	309.98	------	----	0.26	2.28
7	.53	313.76	314.32	0.56	0.82	7.19
6	.95	310.30	312.84	2.54	3.36	29.47
5	1.70	308.56	311.53	2.97	6.33	55.53
4	2.65	311.55	315.02	3.47	9.80	85.96
3	4.40	309.18	310.71	1.53	11.33	99.39
2	11.0	357.86	357.93	0.07	11.40	100
1	18.0	313.48	------	----	-----	-----

Stage	Dp50	Tare Wt. mg	Sample Wt.	mg	Cumul. Tot.	Cumul. %
F	---	106.33	106.62	0.29	0.29	2.22
10	----------	----------	not used	----------	----------	----------
9	.16	314.29	314.41	0.12	0.41	3.14
8	.32	310.01	310.19	0.18	0.59	4.52
7	.53	310.66	311.43	0.77	1.36	10.42
6	.95	311.32	314.12	2.80	4.16	31.88
5	1.70	309.34	312.42	3.08	7.24	55.48
4	2.65	312.12	315.93	3.81	11.05	84.67
3	4.40	312.06	313.86	1.80	12.85	98.47
2	11.0	313.33	313.47	0.14	12.99	99.54
1	18.0	355.64	355.70	0.06	13.05	100

Particle Size Sample Data of Synthetic Graphite Taken During Calibration

Stage	Dp50	Tare Wt. mg	Sample Wt.	mg	Cumul. Tot.	Cumul. %
F	---	120.42	120.45	0.03	0.03	0.27
10	----------	----------	not used	----------	----------	----------
9	.16	304.97	------	----	0.03	0.27
8	.32	314.39	314.45	0.06	0.09	0.81
7	.53	309.48	309.51	0.03	0.12	1.08
6	.95	312.44	314.00	1.56	1.68	15.15
5	1.70	313.52	316.72	3.20	4.88	44.00
4	2.65	315.06	319.73	4.67	9.55	86.11
3	4.40	311.62	313.09	1.47	11.02	99.37
2	11.0	357.69	357.73	0.04	11.06	99.73
1	18.0	354.99	355.02	0.03	11.09	100

Stage	Dp50	Tare Wt. mg	Sample Wt.	mg	Cumul. Tot.	Cumul. %
F	---	120.06	------	----	----	----
10	----------	----------	not used	----------	----------	----------
9	.16	311.63	------	----	----	----
8	.32	312.34	------	----	----	----
7	.53	308.69	309.27	0.58	0.58	4.86
6	.95	309.76	312.38	2.62	3.20	26.82
5	1.70	352.70	355.92	3.22	6.42	53.81
4	2.65	308.51	312.86	4.35	10.77	90.28
3	4.40	308.32	309.48	1.16	11.93	100
2	11.0	311.78	------	----	-----	-----
1	18.0	310.74	------	----	-----	-----

Particle Size Sample Data of Synthetic Graphite Taken During Exposure

Stage	Dp50	Tare Wt. mg	Sample Wt.	mg	Cumul. Tot.	Cumul. %
F	---	119.39	------	----	----	----
10	----------	----------	not used	----------	----------	----------
9	.16	308.93	309.01	0.08	0.08	0.84
8	.32	314.49	314.59	0.10	0.18	1.90
7	.53	358.99	359.45	0.46	0.64	6.76
6	.95	310.55	312.72	2.17	2.81	29.67
5	1.70	308.80	311.82	3.02	5.83	61.56
4	2.65	309.40	311.66	2.26	8.09	85.43
3	4.40	309.13	310.51	1.38	9.47	100
2	11.0	312.28	------	----	----	-----
1	18.0	308.39	------	----	----	-----

Stage	Dp50	Tare Wt. mg	Sample Wt.	mg	Cumul. Tot.	Cumul. %
F	---	120.45	120.67	0.22	0.22	1.01
10	----------	----------	not used	----------	----------	----------
9	.16	304.10	304.34	0.24	0.46	2.11
8	.32	309.38	309.71	0.33	0.79	3.62
7	.53	310.15	311.44	1.29	2.08	9.54
6	.95	312.89	318.68	5.79	7.87	36.10
5	1.70	308.59	314.49	5.90	13.77	63.17
4	2.65	309.20	314.40	5.20	18.97	87.02
3	4.40	350.74	353.38	2.64	21.61	99.13
2	11.0	308.53	308.65	0.12	21.73	99.68
1	18.0	311.13	311.20	0.07	21.80	100

Particle Size Sample Data of TiO2 Taken During Calibration

Stage	Dp50	Tare Wt. mg	Sample Wt.	mg	Cumul. Tot.	Cumul. %
F	---	116.93	117.09	0.16	0.16	0.90
10	----------	----------	not used	----------	----------	----------
9	.16	303.73	304.10	0.37	0.53	2.97
8	.32	312.79	313.90	1.11	1.64	9.19
7	.53	312.66	313.83	1.17	2.81	15.74
6	.95	308.38	312.16	3.78	6.59	36.92
5	1.70	357.93	360.81	2.88	9.47	53.05
4	2.65	311.63	316.50	4.87	14.34	80.34
3	4.40	314.47	317.84	3.37	17.71	99.22
2	11.0	310.57	310.71	0.14	17.85	100
1	18.0	355.96	------	----	-----	-----

Stage	Dp50	Tare Wt. mg	Sample Wt.	mg	Cumul. Tot.	Cumul. %
F	---	119.17	119.37	0.20	0.20	1.65
10	----------	----------	not used	----------	----------	----------
9	.16	311.63	------	----	0.20	1.65
8	.32	312.34	------	----	0.20	1.65
7	.53	308.27	308.87	0.60	0.80	6.60
6	.95	356.24	360.16	3.92	4.72	38.91
5	1.70	304.35	307.85	3.50	8.22	67.77
4	2.65	310.96	313.51	2.55	10.77	88.79
3	4.40	314.00	315.36	1.36	12.13	100
2	11.0	359.39	------	----	-----	-----
1	18.0	315.08	------	----	-----	-----

Particle Size Sample Data of TiO2 Taken During Exposure

Stage	Dp50	Tare Wt. mg	Sample Wt.	mg	Cumul. Tot.	Cumul. %
F	---	120.46	120.55	0.09	0.09	0.95
10	----------	----------	not used	----------	----------	----------
9	.16	303.66	303.84	0.18	0.27	2.85
8	.32	350.45	351.01	0.56	0.83	8.77
7	.53	310.13	311.02	0.89	1.72	18.18
6	.95	311.25	315.16	3.91	5.63	59.51
5	1.70	311.99	313.76	1.77	7.40	78.22
4	2.65	314.25	316.00	1.75	9.15	96.72
3	4.40	312.81	313.12	0.31	9.46	100
2	11.0	310.36	------	----	-----	-----
1	18.0	355.39	------	----	-----	-----

Stage	Dp50	Tare Wt. mg	Sample Wt.	mg	Cumul. Tot.	Cumul. %
F	---	118.77	118.84	0.07	0.07	0.73
10	----------	----------	not used	----------	----------	----------
9	.16	305.70	------	----	0.07	0.73
8	.32	308.15	308.58	0.43	0.50	5.21
7	.53	313.02	313.62	0.60	1.10	11.47
6	.95	309.85	314.24	4.39	5.49	57.25
5	1.70	314.34	316.32	1.98	7.47	77.89
4	2.65	310.41	312.25	1.84	9.31	97.08
3	4.40	313.69	313.97	0.28	9.59	100
2	11.0	352.07	------	----	-----	-----
1	18.0	355.68	------	----	-----	-----

Particle Size Sample Data of TiO2 Taken During Exposure

Stage	Dp50	Tare Wt. mg	Sample Wt.	mg	Cumul. Tot.	Cumul. %
F	---	117.00	117.26	0.26	0.26	2.03
10	----------	----------	not used	----------	----------	----------
9	.16	308.12	308.27	0.15	0.41	3.20
8	.32	311.77	312.61	0.84	1.25	9.77
7	.53	313.01	314.13	1.12	2.37	18.52
6	.95	308.94	314.08	5.14	7.51	58.67
5	1.70	358.55	361.52	2.97	10.48	81.88
4	2.65	313.48	315.37	1.89	12.37	96.64
3	4.40	313.70	314.13	0.43	12.80	100
2	11.0	311.15	------	----	-----	-----
1	18.0	355.37	------	----	-----	-----

Stage	Dp50	Tare Wt. mg	Sample Wt.	mg	Cumul. Tot.	Cumul. %
F	---	100.75	100.87	0.12	0.12	1.05
10	----------	----------	not used	----------	----------	----------
9	.16	308.62	308.81	0.19	0.31	2.70
8	.32	309.41	309.82	0.41	0.72	6.27
7	.53	351.82	352.76	0.94	1.66	14.46
6	.95	312.69	317.19	4.50	6.16	53.66
5	1.70	312.18	315.13	2.95	9.11	79.36
4	2.65	307.32	309.08	1.76	10.87	94.69
3	4.40	358.26	358.79	0.53	11.40	99.30
2	11.0	303.63	303.71	0.08	11.48	100
1	18.0	310.31	------	----	-----	-----

APPENDIX D. ANIMAL HEALTH AND WEIGHT DATA

Blank

DISPOSITION FORM

For use of this form, see AR 340-15; the proponent agency is TAGO.

REFERENCE OR OFFICE SYMBOL	SUBJECT
SMCCR-RST-V	Animal Health Statement for Study #22087000A217

TO Dr. S. Thomson FROM C, Vet Svcs Br. DATE 14 Dec 87 CMT 1

1. I have examined 128 Fisher 344 Rats for study #22087000A217. Of these rats, 2 (#404 and #496) have been found to have sialodacryoadenitis (SDA).

2. SDA has no effect on the respiratory system and therefore will not jeopardize the outcome of this study. Enclosure 1 is a copy of page 284 and 286 from The Laboratory Rat, Volume 1, Biology and Diseases, regarding SDA.

3. The remaining 126 rats have been found in good health.

Stanley P. Liebenberg /ckb

STANLEY P. LIEBENBERG
LTC, VC
Chief, Veterinary Services Branch

DA FORM AUG 80 2496 PREVIOUS EDITIONS WILL BE USED GPO : 1987 O - 172-428

(133). They have physical and chemical characteristics typical of the Herpetoviridae, including a penchant for latency. As with other cytomegaloviruses, rat cytomegalovirus appears to have a predilection for salivary glands and has also been found in lacrimal glands (87). Typical lesions include cytomegaly, intranuclear inclusion body formation in acinar or ductal epithelium, and mild nonsuppurative interstitial inflammation (79,87,128). Kuttner and Wang (79) also showed that salivary glands of affected wild rats contained infectious virus by transmitting disease by intraglandular and ic inoculation of several "young" rats with emulsions of submaxillary gland.

The epizootiology of rat cytomegalovirus has not been well studied, but virus and/or antibody have been detected in wild rats in several widely separated areas of the world (79,128). The potential the virus has for interfering with research has not been evaluated. Infection is usually diagnosed by histological examination. Anti-viral antibody can be detected by NT test (128), but rapid serological tests are not available. Rat cytomegalovirus can be grown in primary rat fibroblasts, rat kidney cells, and hamster kidney cells (6,128).

C. DNA Virus Which May Infect Rats

Mouse Adenovirus

We have found that some rat sera contain CF antibodies to mouse adenovirus, but clinical disease or lesions attributable to infection of rats with this virus have not been detected, and virus has not been recovered from rats.

III. RNA VIRUSES

A. Coronaviruses (Sialodacryoadenitis Virus and Rat Coronavirus)

1. General

Coronaviruses are lipid solvent-labile, pleomorphic, 60 to 220 nm particles with characteristic clublike projections (corona) uniformly arranged on their surfaces. They multiply in the cytoplasm and mature by budding through endoplasmic reticulum. Coronaviridae are fairly species specific and have been identified as etiologic agents in diseases of humans, pigs, bovines, rats, mice, dogs, chickens, and turkeys. They generally infect the gastrointestinal tract and its associated glandular organs and/or the respiratory tract. Reviews of coronavirus biology are available (17,103). Two strains of coronavirus have been identified as important pathogens of laboratory rats: sialodacryoadenitis virus (SDAV) (15) and rat coronavirus (RCV) (120).

2. History

In 1961, Innes and Stanton reported two outbreaks of clinical disease in weanling rats characterized by cervical edema and "red tears" (59). They described the morphology of the disease in considerable detail and named it sialodacryoadenitis from the characteristic lesions: inflammation and edema of salivary and lacrimal glands. Hunt (58) described a similar disease of young rats, but inflammation was restricted to the intraorbital lacrimal glands and was accompanied by keratoconjunctivitis. Innes and Stanton suggested an infectious agent caused the disease, and Hunt detected acidophilic intranuclear inclusion bodies in affected Harderian glands and in conjunctival mucosa, but viral isolations were not attempted in either study. Ashe and co-workers (3,4) isolated a transmissible cytopathic viral agent from the submaxillary glands of gnotobiotic rats that hemagglutinated rabbit erythrocytes. Ashe's virus apparently was not associated with clinical signs or lesions in infected rats (see Section IV,B). Jonas *et al.* (67) induced sialodacryoadenitis in germfree rats by intranasal inoculation of an ultrafiltrate of diseased submaxillary salivary gland. Virus particles were detected in ducts of submaxillary glands from experimentally infected rats by electron microscopy, but attempts to isolate an agent *in vitro* were initially unsuccessful. However, when neonatal mice were inoculated ic with submaxillary gland homogenate they developed severe neurological deficits and died in 3 to 6 days. Brain homogenates from affected mice caused sialodacryoadenitis in intranasally inoculated rats. Bhatt and co-workers (15) subsequently isolated a virus from salivary glands of affected rats by inoculation of neonatal mice and primary rat kidney (PRK) monolayer cultures. The isolate had serological and physicochemical characteristics of a coronavirus. It was lethal for infant mice after ic inoculation but not for weanling mice. Mouse brain-passaged virus induced sialodacryoadenitis in susceptible rats.

In 1964 Hartley and associates found that some rat sera contained antibody to mouse hepatitis virus (MHV) (51). They suggested that an agent antigenically related to MHV could elicit anti-MHV antibody in rats. Parker *et al.* (120) offered support for Hartley's theory by isolating a coronavirus antigenically related to MHV from lungs of infected but clinically normal rats. Parker's virus (RCV) was subsequently shown to be antigenically related to both SDAV and MHV (15), and the current view is that SDAV and RCV may be different strains of one rat coronavirus. The biology of each virus is discussed separately in each of the following sections.

3. Viral Characteristics

a. SDAV. The diameter of SDAV particles, determined by ultrafiltration, is 100 to 220 nm. Jonas *et al.* (67) described a 60- to 70-nm particle in ductal epithelium of experimentally

mice developed NT antibody to SDAV, whereas in inoculated mice developed NT and CF antibody. The virus was recovered from the respiratory tract for up to 7 days postinfection, and mice developed interstitial pneumonia. Anti-SDAV and anti-MHV antibody also has been found among retired breeder mice from colonies thought to be free of MHV. Since SDAV and MHV are antigenically related, SDAV infection should be considered if unexpected or unexplained seroconversions to MHV occur in mouse colonies. Seroconversions to MHV from infection of mice or rats exposed to human coronaviruses (e.g., carried by animal technicians) also should be considered (Hartley *et al.*, 1964) but has not been studied.

Extensive host range studies of SDAV have not been done, but preliminary trials with several strains of rats and mice suggest that various strains of SDAV may vary in infectivity and antigenicity (14). For example, during spontaneous outbreaks, WAG/Rij rats developed severe clinical disease, whereas DA rats developed primarily subclinical disease. Furthermore, some strains of mice developed both CF and NT antibody following experimental SDAV infection, whereas others produced only NT antibody. Conversely, one strain of SDAV induced only NT antibody in a given mouse strain whereas a second strain of SDAV induced both CF and SN antibody. These variations are important for interpretation of diagnostic and epizootiological data.

b. RCV. Host range studies with RCV also have been limited. Rat coronavirus is infectious for rats and induces seroconversion to RCV, MHV, and SDAV (11,15,120). Its pathogenicity varies with strain and age but is greatest for suckling rats. For example, mortality among intranasally inoculated Fischer 344 rats less than 48 h old approached 100%, whereas comparable Wistar rats had only 10 to 25% mortality. Furthermore, deaths among Fischer 344 sucklings occurred 6 to 12 days after infection, whereas Wistar sucklings usually died after 12 days. Resistance to mortality, however, among even highly susceptible sucklings, increased rapidly so that rats inoculated after 7 days of age had nonfatal respiratory disease and weanlings were asymptomatic (120). The pathogenicity and infectivity of RCV for other species have not been reported.

6. Clinical Disease

a. SDAV. Susceptible rats can be infected at any age, but clinical disease usually occurs in one of two patterns: endemic infection of breeding colonies or explosive outbreaks among nonimmune rats exposed to virus as weanlings or adults. In the former setting, adults may have clinical signs, but more commonly they are immune. Therefore, clinical disease develops among susceptible sucklings and is characterized by so-called "winking and blinking" associated with acute inflammation of the eye and adnexae. Signs are transient (1 week or less) among individual sucklings, but affected animals will be prevalent among the suckling population as long as newly susceptible litters are available to become infected. In the latter situation, either new, SDAV-susceptible rats are placed in a room with infected rats or an infected rat(s) is placed in a room housing nonimmune weanlings or adults. Generally, within 1 week, the susceptible population will develop typical signs of SDAV infection. For individual rats they include cervical swelling (edema) with palpable enlargement of submaxillary salivary glands, sneezing or repeated wiping of the external nares with the forepaws, photophobia and nasal, and ocular discharges which are often red-tinged due to a high content of porphyrin pigment. Clinical signs last about 1 week. They may be mild or severe, and all signs do not occur in every infected rat. This last point is especially significant, since a single subclinically infected rat placed in a susceptible colony can initiate a full enzootic episode.

Keratoconjunctivitis has been associated with several natural outbreaks of SDAV (80,161) and may be the only clinically detectable evidence of SDAV infection. Signs and lesions commonly begin by the time of weaning, but also can occur in adults. They include photophobia, lacrimation, circumcorneal flush, diffuse corneal opacities, corneal ulcers, pannus, hypopyon, and hyphema. Lesions usually resolve completely in 1 to 2 weeks, but chronic active keratitis and megaloglobus may develop in some rats. The morbidity of eye lesions during an acute outbreak of SDAV infection varies from 0 to 100%, but is usually 10 to 30%. The prevalence of eye lesions seems greater among breeding colonies chronically infected with SDAV (65). The severity of lesions also may vary among strains of rats. In our experience, inbred Lewis and WAG/Rij rats are more susceptible to SDAV-associated eye disease than DA rats or outbred CD rats (65). Weisbroth and Peress (161) found that the spontaneously hypertensive strain TAC:SHR/N also was highly susceptible. The pathology of the eye lesions is discussed in greater detail in Section III, A, 7.

b. RCV. Rat coronavirus infection is subclinical in postweaned rats. Nonfatal respiratory disease can occur in intranasally inoculated sucklings, and intranasally infected susceptible neonates may die (11,120).

7. Pathology

a. SDAV. The lesions of SDAV infection have been described in detail by several groups of workers (59,61,67,80,161).

Gross Lesions of SDAV infection usually are restricted to mixed or serous salivary glands, lacrimal glands, cervical lymph nodes, thymus, and occasionally lung. Submaxillary and parotid salivary glands and cervical lymph nodes are un-

SGRD-UV-VM 26 Feb 88

MEMORANDUM FOR RECORD

SUBJECT: Quality Assurance Data

1. The following information concerns Quality Assurance data for a group of five Fisher Rats, accession numbers 88-0025 through 88-0029.

ACC No.	Microbiology	Parasitology
88-0025	TW-NG GUT-NEPI	NOPS
88-0026	TW-Staph spp -Enterococcus spp. -E.coli GUT-NEPI	NOPS
88-0027	TW-P. mirabilis GUT-NEPI	NOPS
88-0028	TW-Enterococcus spp. GUT-NEPI	NOPS
88-0029	TW-NG GUT-NEPI	NOPS

TW = Trachael Wash; NOPS = No Obvious Parasites Seen
NEPI = No Enteric Pathogens Isolated; NG = No Growth

2. POC for this information is CPT(P) Parrish, ext 3503.

Denver D. Marlow
DENVER D. MARLOW
MAJ, VC
Chief, Vet Med & Surg Br

CF:
C, Vet Med & Lab Resr Div
C, Veterinary Services Branch, CRDEC

GEORGE A. PARKER, DVM, LTD
111-A Carpenter Drive
P.O. Box 764
Sterling, VA 22170
(703)481-1122

Mr. John Graham
US Army Medical Research Institute of Chemical Defense
Comparative Pathology Branch
Aberdeen Proving Ground, MD 21010-5425

Dear Mr. Graham:

Enclosed please find histology worksheets, paraffin blocks, microslides, residual wet tissues and 2 copies of the pathology report from a group of quality control animals received 27Jan88. Animals examined are summarized on the attached letter from yourself dated 27Jan88.

Sincerely,

George A. Parker, DVM, DACVP 29Feb88

DEPARTMENT OF THE ARMY
UNITED STATES ARMY MEDICAL RESEARCH INSTITUTE OF CHEMICAL DEFENSE
ABERDEEN PROVING GROUND, MARYLAND 21010-5425

REPLY TO ATTENTION OF

January 27, 1988

SGRD-UV-YC

Dr. George A. Parker, Ltd
P.O. Box 350
Great Falls, Virginia 22066

Dear Sir:

Reference Contract/Order No. DAAD05-88-C-0021 and letter from H.G. Wall, dated October 3, 1985, the following tissues are submitted for processing and evaluation:

DATE OF NECROPSY	PATHOLOGY ACCESSION NO.	SOURCE	RECEIVED	SPECIES	NUMBER	
Sept 11	87-1134-1138	CR1903440P	Sept 9	Mouse	5	
"	87-1139-1143	CR2929029K	Sept 2	G. Pig	5	Issued
Sept 16	87-1147-1151	CR1903440P	Sept 9	Mouse	5	Issued
Sept 18	87-1153-1156	CR1991093P	Sept 16	Rat	4	
Sept 25	87-1163-1166	CR2722817K	Sept 24	Mouse (Retired)	4	
"	87-1167-1169	CR2722818K	"	Rat (Retired)	3	
Sept 30	87-1172-1176	CR1991093P	Sept 16	Rat	5	Issued
"	87-1177-1181	CR2929020K	Sept 23	G. Pig	5	
Oct 2	87-1183-1187	CR1903440P	Sept 30	Mouse	5	
"	87-1188-1192	CR2929029K	Sept 23	G. Pig	5	Issued
Oct 7	87-1201-1205	CR1903440P	Sept 30	Mouse	5	Issued
Oct 9	87-1210-1214	CR2929020K	Oct 7	G. Pig	5	
Oct 14	87-1218	Hazelton	Oct 13	Rabbit	1	
"	87-1219-1223	CR2929029K	Oct 7	G. Pig	5	Issued
Oct 16	87-1225-1229	CR1001169P	Oct 14	Rat	5	
Oct 21	87-1232-1236	CR1001169P	"	Rat	5	Issued
Oct 28	87-1252-1256	CR1903440P	Oct 21	Mouse	5	
"	87-1257-1261	CR1903440P	"	Mouse	5	Issued
Oct 30	87-1266-1268	CR2722818K	Oct 28	Rat (Retired)	3	
"	87-1269-1272	CR2722817K	"	Mouse (Retired)	4	
"	87-1273-1277	CR2929020K	"	G. Pig	5	
Nov 4	87-1291-1295	CR2929029K	Oct 28	G. Pig	5	Issued
Nov 6	87-1300-1304	CR1903440P	Nov 4	Mouse	5	
Nov 13	87-1334-1338	CR1903440P	"	Mouse	5	Issued
Nov 18	87-1354-1358	CR2929020K	Nov 11	G. Pig	5	
"	87-1359-1363	CR2929029K	"	G. Pig	5	Issued
Nov 20	87-1368	Hazelton	Nov 17	Rabbit	1	Issued
"	87-1369-1373	CR1903440P	Nov 18	Mouse	5	
Nov 25	87-1376-1380	CR1903440	"	Mouse	5	Issued
Dec 2	87-1387-1389	CR2722818K	Nov 25	Rat (Retired)	3	
"	87-1390-1393	CR2722817K	"	Mouse (Retired)	4	
Dec 4	87-1394-1398	CR1903440P	Dec 2	Mouse	5	
"	87-1399-1403	CR2929029K	Nov 25	G. Pig	5	
"	87-1404-1407	CR2929029K	"	G. Pig	4	Issued

DATE OF NECROPSY	PATHOLOGY ACCESSION NO.	SOURCE	RECEIVED	SPECIES	NUMBER
Dec 9	87-1422-1426	CR1903440P	Dec 2	Mouse	5 Issued
Dec 11	87-1431-1435	CR1903440P	Dec 9	Mouse	5
Dec 16	87-1437-1441	CR1903440P	"	Mouse	5 Issued
Jan 4	88-0001-0004	CR2722817K	Dec 23	Mouse (Retired)	4
"	88-0005-0007	CR2722818K	"	Rat (Retired)	3
"	88-0008-0012	CR2929020K	Dec 23	G. Pig	5
"	88-0013-0017	CR2929029K	"	G. Pig	5 Issued
Jan 6	88-0019	Hazelton	Jan 5	Rabbit	1
"	88-0020-0024	CR1026572P	Dec 30	Rat	5 Issued
"	88-0025-0029	CRDEC	Nov 17	Rat	5
Jan 7	88-0032-0035	CR1903440P	Jan 6	Mouse	4
"	88-0039	CR1903440P	Jan 6	Mouse	1
Jan 13	88-0056	Hazelton	Jan 5	Rabbit	1 Issued
"	88-0057-0061	CR1903440P	Jan 6	Mouse	5 Issued
Jan 15	88-0071-0075	CR2929020K	Jan 13	G. Pig	5
Jan 20	88-0088-0092	CR2929029K	"	G. Pig	5 Issued
"	88-0093	Hazelton	Jan 19	Rabbit	1
Jan 22	88-0110-0114	CR1903440P	Jan 20	Mouse	5
"	88-0115	Hazelton	Jan 19	Rabbit	1 Issued
				TOTAL	222

Please feel free to contact me at 301-671-3389 for any further information required in processing/evaluation. Reports are due my office at your convenience.

Sincerely,

John S. Graham
Contracting Representative
Comparative Pathology Branch

ACKNOWLEDGE RECEIPT OF TISSUES

1.25

DATE: 1/27/88

INDIVIDUAL ANIMAL PATHOLOGY REPORT

SPONSOR: USAMRICD SHIPMENT NO.: 1 PATH. NO.: 88-0025

SPECIES: Rat SOURCE: CRDEC ANIMAL NO.: 1

BODY WEIGHT: 306

GROSS OBSERVATIONS:
Liver slightly fat.

MICROSCOPIC FINDINGS:
Tongue- vascular mineralization, moderate

TISSUES MISSING: none

COMMENT:
The vascular mineralization in the tongue was considered to be an incidental finding, of no significance to colony management. There was no microscopic correlate of the gross observation in the liver.

INDIVIDUAL ANIMAL PATHOLOGY REPORT

SPONSOR: USAMRICD SHIPMENT NO.: 1 PATH. NO.: 88-0026

SPECIES: Rat SOURCE: CRDEC ANIMAL NO.: 2

GROSS OBSERVATIONS: NSL BODY WEIGHT: 318

MICROSCOPIC FINDINGS:
Essentially normal tissues

MISSING TISSUES: 1 adrenal

INDIVIDUAL ANIMAL PATHOLOGY REPORT

SPONSOR: USAMRICD SHIPMENT NO.: 1 PATH. NO.: 88-0027

SPECIES: Rat SOURCE: CRDEC ANIMAL NO.: 3

BODY WEIGHT: NS

GROSS OBSERVATIONS:
3x3x2 mm oval shaped mass, hard, attached to peritoneal fat

MICROSCOPIC FINDINGS:
Peritoneal cavity- fat necrosis, focal, moderate

MISSING TISSUES: mandibular lymph node, 1 adrenal

COMMENT:
Fat necrosis in the peritoneal cavity is a common incidental finding, presumably a result of strangulation of a small mass of normal fatty tissue. It is considered to be of no significance to colony management.

INDIVIDUAL ANIMAL PATHOLOGY REPORT

SPONSOR: USAMRICD SHIPMENT NO.: 1 PATH. NO.: 88-0028

SPECIES: Rat SOURCE: CRDEC ANIMAL NO.: 4

GROSS OBSERVATIONS: NSL BODY WEIGHT: 337

MICROSCOPIC FINDINGS:
Essentially normal tissues

MISSING TISSUES: none

INDIVIDUAL ANIMAL PATHOLOGY REPORT

SPONSOR: USAMRICD SHIPMENT NO.: 1 PATH. NO.: 88-0029

SPECIES: Rat SOURCE: CRDEC ANIMAL NO.: 5

GROSS OBSERVATIONS: NSL BODY WEIGHT: 32

MICROSCOPIC FINDINGS:
Essentially normal tissues

MISSING TISSUES: 1 adrenal, Harderian glands

BODY WEIGHT SUMMARY
COMPARATIVE ACUTE INHALATION SCREEN OF TITANIUM OXIDE AND GRAPHITE DUSTS
PROTOCOL-22087000A217

		SYNTHETIC GRAPHITE	NATURAL GRAPHITE	TITANIUM OXIDE	CONTROLS
11-13-87	x	245.16	247.19	247.22	246.09
	s	8.95	7.47	9.13	9.41
11-20-87	x	259.28	263.06	264.38	260.00
	s	10.05	7.69	9.40	9.48
11-28-87	x	274.81	276.19	279.53	275.06
	s	9.67	8.42	10.96	9.80
12-04-87	x	285.56	289.63	291.16	287.44
	s	12.20	8.43	12.02	10.13
12-11-87	x	291.25	296.09	297.19	290.63
	s	12.24	8.86	12.22	11.54
12-13-87	x	292.09	293.31	295.38	287.69
	s	12.15	9.35	12.34	10.37
12-14-87	x	289.47	292.91	293.91	288.41
	s	12.48	9.05	12.29	10.59
12-15-87	x	289.09	291.34	292.66	287.38
	s	12.48	8.63	12.93	10.86
12-16-87	x	288.69	291.31	292.16	288.03
	s	12.49	9.10	12.27	10.71
12-18-87	x	290.69	287.44	295.06	291.50
	s	14.11	8.24	7.31	13.11
12-25-87	x	298.44	299.31	304.44	297.19
	s	14.88	11.01	6.12	13.40

DATA ANALYSIS - BODY WEIGHTS OF MALE FISCHER 344 RATS
PROTOCOL-22087000A217

	BARTLETT'S TEST				ANOVA(oneway)
	SYNTHETIC GRAPHITE	NATURAL GRAPHITE	TITANIUM OXIDE	CONTROL	SYNTHETIC GRAPHITE
11-13-87	*NS*	*NS*	*NS*	*NS*	
11-20-87	*NS*	*NS*	*NS*	*NS*	
11-27-87	*NS*	*NS*	*NS*	*NS*	
12-04-87	*NS*	*NS*	*NS*	*NS*	
12-11-87	*NS*	*NS*	*NS*	*NS*	
12-13-87	*NS*	*NS*	*NS*	*NS*	
12-14-87	*NS*	*NS*	*NS*	*NS*	
12-15-87	*NS*	*NS*	*NS*	*NS*	
12-16-87	*NS*	*NS*	*NS*	*NS*	
12-18-87	*NS*	*NS*	*NS*	*NS*	
12-25-87	*SIG*	*NS*	*NS*	*NS*	*NS*

WEIGHT TABLE 1 CONTROL GROUP
13 NOVEMBER 1987 - 25 DECEMBER 1987

ID#	11/13	11/20	11/28	12/04	12/11	12/13	12/14	12/15	12/16	12/18	12/25
400	237	252	269	281	282	280	280	281	281		
401	252	264	276	290	289	285	285	285	288		
402	235	254	268	283	284	282	281	276	277		
403	254	261	276	293	293	288	290	286	288		
404	238	253	268	284	283	277	277	277	278		
405	264	276	291	309	313	311	307	304	304		
406	254	263	279	289	294	294	296	296	295		
407	232	249	266	282	283	286	287	285	284		
408	247	256	269	275	278	279	280	277	281		
409	254	273	290	294	302	295	296	295	297		
410	235	247	263	275	277	279	280	278	280		
411	256	266	279	289	297	294	296	297	297		
412	255	273	284	300	303	298	302	300	301		
413	260	272	287	297	304	301	300	301	301		
414	234	246	260	272	270	268	271	272	272		
416	245	262	273	286	286	288	289	287	285		
415	254	269	285	297	302	300	298	299	298	302	294
418	251	264	280	283	294	292	293	296	294	295	303
419	249	266	284	289	299	293	291	294	293	297	300
420	237	256	269	284	288	280	283	283	283	283	283
421	234	254	266	280	283	281	282	281	281	283	289
422	230	238	250	264	265	262	262	260	260	262	269
423	252	264	281	294	298	294	295	291	293	295	303
424	244	261	279	291	295	296	295	295	293	296	299
425	241	256	273	288	292	292	293	293	294	299	304
426	238	250	270	281	285	280	280	280	282	287	299
427	251	263	273	282	281	282	284	283	284	286	292
428	257	269	287	303	307	302	304	303	305	313	318
429	250	264	279	290	295	284	282	280	283	291	308
430	251	266	280	293	294	289	289	286	288	286	291
431	251	270	288	307	310	302	307	304	306	314	322
432	233	243	260	273	274	272	274	271	271	275	281
x	246.09	264.15	279.12	287.50	287.50	287.91	288.41	287.38	288.03	291.50	297.19
s	9.41	6.79	7.21	10.04	10.04	10.46	10.59	10.86	10.71	13.11	13.40

WEIGHT TABLE 2 SYNTHETIC GRAPHITE EXPOSURE GROUP
13 NOVEMBER 1987 - 25 DECEMBER 1987

ID#	11/13	11/20	11/28	12/04	12/11	12/13	12/14	12/15	12/16	12/18	12/25
433	244	254	269	277	280	276	275	273	271		
434	253	271	287	304	310	307	301	301	297		
435	248	260	275	285	294	293	290	292	291		
436	253	262	277	288	297	295	293	290	288		
437	229	245	263	267	276	273	272	271	267		
438	243	258	270	281	284	284	281	285	285		
439	239	250	265	275	278	278	276	276	279		
440	245	253	265	274	281	278	277	275	274		
441	258	272	292	307	313	306	307	307	307		
442	250	263	283	301	310	301	300	298	297		
443	235	254	272	288	302	299	300	301	299		
444	235	250	269	281	282	278	279	276	277		
445	245	259	273	285	290	288	288	285	284		
446	248	265	284	291	306	306	303	300	301		
447	247	263	283	299	307	296	295	297	297		
448	244	258	270	282	289	289	289	287	285		
449	234	244	260	267	274	275	271	272	272	272	279
450	238	252	271	277	284	284	283	283	284	284	295
451	247	264	278	284	289	292	289	287	288	287	300
452	239	257	277	283	292	290	291	290	293	292	294
453	242	263	276	290	295	295	296	297	298	299	301
454	243	258	275	288	295	297	293	294	294	296	301
455	247	261	275	287	295	295	294	293	293	294	305
456	248	263	278	291	288	293	294	291	292	293	308
457	229	240	253	267	272	271	262	265	264	265	270
458	259	276	288	304	308	306	301	304	301	306	317
459	232	248	261	269	278	276	275	275	274	274	282
460	259	273	285	292	304	307	304	306	306	307	313
461	242	255	264	267	275	276	273	273	273	273	279
462	246	258	274	282	290	293	293	292	295	295	302
463	262	274	285	292	306	306	303	300	298	298	304
464	262	283	297	313	303	317	315	315	314	316	325
x	245.16	259.56	274.81	285.56	292.09	291.25	289.47	289.09	288.69	290.69	298.44
s	8.95	9.76	9.67	12.20	12.15	12.24	12.48	12.48	12.49	14.11	14.88

WEIGHT TABLE-3 NATURAL GRAPHITE EXPOSURE GROUP
13 NOVEMBER 1987- 25 DECEMBER 1987

ID#	11/13	11/20	11/28	12/04	12/11	12/13	12/14	12/15	12/16	12/18	12/25
465	242	259	271	283	292	291	289	285	286		
466	259	273	280	297	309	309	309	304	304		
467	243	256	267	277	286	287	285	286	287		
468	253	270	286	298	301	297	293	291	296		
469	256	265	283	296	306	303	298	297	297		
470	249	264	285	289	300	302	299	300	300		
471	236	249	262	274	279	281	279	279	274		
472	260	274	288	299	305	305	304	302	301		
473	243	261	277	291	296	294	294	294	293		
474	254	272	284	295	307	308	307	304	306		
475	248	263	273	285	294	293	295	290	291		
476	244	255	270	285	293	290	291	294	296		
477	248	265	278	291	301	297	299	300	301		
478	251	268	287	302	309	308	306	303	302		
479	238	258	263	287	297	296	294	293	292		
480	247	263	272	285	295	294	293	291	291		
481	232	244	258	265	274	268	268	268	266	265	277
482	244	264	282	293	295	288	287	287	285	286	300
483	248	269	284	297	304	298	302	300	299	295	304
484	247	260	275	289	294	292	291	290	289	290	299
485	241	254	267	279	280	277	278	278	276	278	285
486	260	274	281	296	301	297	298	297	297	296	305
487	244	263	271	286	292	285	289	288	287	281	294
488	244	259	265	290	292	290	287	285	284	286	293
489	241	259	270	287	291	293	293	290	288	291	301
490	258	276	284	293	300	296	297	291	293	293	303
491	247	271	283	295	294	288	289	286	287	288	325
492	248	259	274	290	291	289	290	287	291	286	296
493	260	273	291	307	312	307	305	305	303	299	313
494	245	264	278	295	302	293	294	290	291	292	305
495	245	261	278	287	296	290	290	290	289	291	299
496	235	253	271	285	287	280	280	278	280	282	290
x	247.19	263.06	276.19	296.09	289.63	293.31	291.91	291.34	291.31	287.41	299.31
std	7.47	7.69	8.42	8.86	8.43	9.35	9.05	8.63	9.10	8.24	11.01

WEIGHT TABLE 4 TITANIUM OXIDE EXPOSURE GROUP
13 NOVEMBER 1987 - 25 DECEMBER 1987

ID#	11/13	11/20	11/28	12/04	12/11	12/13	12/14	12/15	12/16	12/18	12/25
497	283	301	324	341	352	351	350	352	349		
498	229	244	259	271	282	280	277	274	278		
499	247	268	285	292	293	293	294	293	290		
500	255	270	288	297	293	302	300	298	300		
501	250	269	285	298	305	304	303	302	299		
502	240	260	277	292	295	291	292	290	289		
503	254	264	275	290	296	293	292	295	292		
504	251	268	284	297	301	302	302	302	302		
505	239	254	269	276	289	285	285	283	287		
506	245	263	278	291	300	298	295	293	293		
507	246	264	282	291	296	297	295	293	293		
508	242	256	272	282	284	291	290	288	286		
509	242	264	280	285	295	291	293	292	288		
510	243	255	265	280	280	284	280	281	279		
511	252	269	283	300	300	303	298	296	297		
512	244	262	278	292	296	294	295	294	291		
513	247	269	282	296	300	292	293	293	295	304	315
514	248	263	280	289	293	292	287	285	285	288	298
515	252	268	281	296	307	305	299	301	299	306	311
516	258	272	289	299	300	297	290	292	290	295	301
517	251	263	273	282	292	285	287	285	284	288	295
518	252	274	292	306	307	302	302	300	299	304	313
519	241	263	280	292	296	289	288	287	286	283	305
520	240	257	273	281	287	283	282	282	283	293	297
521	245	259	270	279	289	287	285	285	286	297	309
522	240	260	279	286	297	292	291	288	287	293	303
523	258	276	286	301	312	310	307	306	305	309	310
524	242	258	273	284	293	292	287	287	290	295	302
525	243	263	270	287	296	292	289	288	287	294	308
526	252	269	286	292	296	294	294	290	287	292	300
527	240	256	270	285	290	287	288	283	282	288	298
528	240	259	277	287	298	294	295	287	291	292	306
x	247.22	263.59	277.94	291.16	297.19	295.38	293.34	292.66	292.16	295.06	304.44
std	9.13	10.33	11.83	12.02	12.22	12.34	12.03	12.93	17.06	7.31	6.12

ORGAN/BODY RATIOS OF MALE FISCHER 344 RATS EXPOSED TO SYNTHETIC GRAPHITE, NATURAL GRAPHITE, AND TITANIUM OXIDE

24 HOUR POST EXPOSURE

CONTROL

ANIMAL NUMBER	BODY WT (GMS.)	ADRENALS $x10^{-4}$	BRAIN $x10^{-3}$	HEART $x10^{-3}$	KIDNEYS $x10^{-3}$	LIVER $x10^{-2}$	LUNGS $x10^{-3}$	TESTES $x10^{-2}$
400	280.4	2.07	6.53	3.21	8.45	4.13	4.39	1.06
401	288.4	2.25	6.21	3.22	8.60	4.37	4.06	1.03
402	279.8	1.82	6.65	3.43	8.33	4.09	4.43	1.05
403	292.2	2.57	6.16	3.35	8.49	4.36	3.83	0.97
404	279.0	1.51	6.60	3.26	8.39	4.29	4.23	1.03
405	306.5	2.09	6.39	3.39	8.97	4.24	4.25	1.09
406	295.9	2.30	6.49	3.24	8.25	4.34	4.93	0.96
407	284.3	1.90	6.58	3.34	7.84	4.07	4.43	1.06
408	278.8	2.19	6.60	3.26	7.96	4.10	4.34	0.95
409	298.1	2.08	6.27	3.29	8.52	4.16	3.59	0.97
x	288.34	2.08	6.45	3.30	8.38	4.22	4.25	1.02
std.dev.	9.59	0.29	0.18	0.07	0.32	0.12	0.37	0.05

SYNTHETIC GRAPHITE

ANIMAL NUMBER	BODY WT. (GMS.)	ADRENALS $x10^{-4}$	BRAIN $x10^{-3}$	HEART $x10^{-3}$	KIDNEYS $x10^{-3}$	LIVER $x10^{-2}$	LUNGS $x10^{-3}$	TESTES $x10^{-2}$
433	272.0	1.88	6.51	2.98	8.27	4.63	4.26	0.98
434	294.3	2.17	6.49	3.26	8.05	4.23	4.89	1.02
435	286.6	2.02	6.63	3.14	7.92	3.99	5.06	1.00
436	284.9	1.97	6.60	3.19	8.11	3.76	4.53	1.02
437	266.1	1.84	6.76	3.42	7.93	3.77	4.69	1.15
438	284.5	1.83	6.43	3.06	8.19	4.45	4.75	0.99
439	275.4	2.40	6.79	3.30	8.39	4.83	4.87	1.08
440	273.2	1.79	6.00	3.37	9.15	4.03	4.98	0.99
441	305.1	2.03	6.19	3.38	8.06	4.15	4.33	0.99
442	296.3	2.02	6.38	3.41	8.34	5.05	4.59	1.03
x	283.84	2.00	6.48	3.25	8.24	4.29	4.70	1.03
std.dev	12.32	1.84	0.24	0.15	0.36	0.44	0.27	0.05

ORGAN/BODY RATIOS OF MALE FISCHER 344 RATS EXPOSED TO SYNTHETIC GRAPHITE, NATURAL GRAPHITE, AND TITANIUM OXIDE

24 HOUR POST EXPOSURE

NATURAL GRAPHITE

ANIMAL NUMBER	BODY WT. (GMS.)	ADRENALS $x10^{-3}$	BRAIN $x10^{-3}$	HEART $x10^{-3}$	KIDNEYS $x10^{-3}$	LIVER $x10^{-2}$	LUNGS $x10^{-3}$	TESTES $x10^{-2}$
465	281.7	2.50	6.67	3.48	8.27	4.12	4.76	1.02
466	304.7	2.10	6.20	3.05	7.75	4.13	5.55	1.03
467	284.2	1.97	6.40	3.24	8.09	3.88	4.75	1.02
468	293.8	1.94	6.43	3.34	8.07	4.16	4.96	1.03
469	297.0	1.58	6.40	3.13	8.18	4.20	4.16	1.05
470	296.4	2.02	6.31	3.31	7.96	4.43	4.52	1.04
471	273.7	2.23	6.69	3.29	7.53	3.87	5.52	1.15
472	301.3	1.66	6.27	3.22	7.73	4.08	5.01	1.05
473	290.3	2.14	6.54	3.03	8.10	4.11	4.34	1.10
474	304.5	2.04	6.01	3.12	7.88	4.39	5.19	0.99
x	292.76	2.12	6.39	3.22	7.96	4.14	4.92	1.05
std.dev.	10.28	0.40	0.21	0.14	0.23	0.18	0.41	0.01

TITANIUM OXIDE

ANIMAL NUMBER	BODY WT. (GMS.)	ADRENALS $x10^{-4}$	BRAIN $x10^{-3}$	HEART $x10^{-3}$	KIDNEYS $x10^{-3}$	LIVER $x10^{-2}$	LUNGS $x10^{-3}$	TESTES $x10^{-2}$
497	354.7	2.20	5.44	3.07	8.18	4.96	4.31	0.91
498	377.3	2.31	6.67	3.28	7.86	4.08	4.58	0.91
499	293.7	2.32	6.37	3.20	8.04	4.28	4.60	1.07
500	300.4	2.03	6.19	2.20	7.56	4.32	5.23	1.04
501	302.2	1.65	6.12	2.98	7.91	4.04	4.47	1.03
502	290.4	1.76	6.37	3.17	7.58	4.14	4.44	0.95
503	292.3	2.53	6.29	3.11	8.18	4.39	4.24	1.03
504	303.2	1.81	6.56	3.27	7.78	3.78	5.15	0.96
505	290.3	2.07	6.37	3.20	7.82	2.90	4.44	1.01
506	297.4	1.75	6.32	3.26	7.83	4.70	4.64	1.02
x	300.19	2.04	6.27	3.17	7.87	4.16	4.61	1.00
std.dev.	20.59	0.30	0.33	0.10	0.21	0.56	0.33	0.05

ORGAN/BODY RATIOS OF MALE FISCHER 344 RATS EXPOSED TO SYNTHETIC GRAPHITE, NATURAL GRAPHITE, AND TITIANIUM OXIDE

14 DAY POST EXPOSURE

CONTROL

ANIMAL NUMBER	BODY WT. (GMS.)	ADRENALS $x10^{-4}$	BRAIN $x10^{-3}$	HEART $x10^{-3}$	KIDNEYS $x10^{-3}$	LIVER $x10^{-2}$	LUNGS $x10^{-3}$	TESTES $x10^{-2}$
415	313.30	1.83	5.67	2.71	7.24	3.58	3.59	0.90
418	307.50	1.14	6.10	2.94	7.22	3.55	4.09	0.98
419	302.90	1.16	6.09	2.83	7.69	3.80	4.00	0.98
420	289.10	1.27	6.36	2.71	7.33	3.42	4.28	1.01
421	293.40	1.25	6.27	2.43	6.94	3.12	3.81	0.96
422	276.50	1.25	6.55	2.86	7.05	3.60	3.95	1.08
423	306.20	1.48	6.34	3.20	8.09	4.12	4.51	1.02
424	311.00	1.23	6.01	2.53	7.19	3.78	4.12	0.93
425	314.80	1.53	5.75	2.59	7.31	3.60	3.65	0.96
426	303.70	1.38	5.95	2.84	7.46	4.22	3.13	0.95
x	301.84	1.35	6.11	2.76	7.35	3.68	3.91	0.98
std.dev.	12.09	0.21	0.28	0.22	0.33	0.32	0.39	0.05

SYNTHETIC GRAPHITE

ANIMAL NUMBER	BODY WT. (GMS.)	ADRENALS $x10^{-4}$	BRAIN $x10^{-3}$	HEART $x10^{-3}$	KIDNEYS $x10^{-3}$	LIVER $x10^{-2}$	LUNGS $x10^{-3}$	TESTES $x10^{-2}$
449	280.40	1.52	6.63	3.25	8.12	3.86	4.07	1.03
450	297.50	1.18	6.26	2.82	7.46	3.47	3.60	0.92
451	306.80	1.40	6.00	2.70	7.08	3.50	4.15	0.95
452	306.40	1.25	6.31	2.62	7.34	3.59	4.48	0.82
453	306.50	1.57	6.20	2.58	7.20	3.40	4.00	0.95
454	306.00	1.45	5.96	2.87	7.26	4.01	3.16	0.94
455	313.90	1.33	6.00	2.71	7.11	3.85	4.18	0.93
456	317.70	1.15	5.88	2.74	7.16	3.65	3.95	0.94
457	277.80	1.35	6.53	2.69	7.88	3.53	3.92	0.98
458	324.80	1.46	5.92	2.73	7.64	3.98	4.17	0.92
x	303.78	1.37	6.17	2.77	7.43	3.68	4.01	0.94
std.dev.	15.01	0.14	0.26	0.19	0.35	0.22	0.27	0.05

ORGAN/BODY RATIOS OF MALE FISCHER 344 RATS EXPOSED TO SYNTHETIC GRAPHITE, NATURAL GRAPHITE, AND TITANIUM OXIDE

14 DAY POST EXPOSURE

NATURAL GRAPHITE

ANIMAL NUMBER	BODY WT. (GMS.)	ADRENALS $x10^{-4}$	BRAIN $x10^{-3}$	HEART $x10^{-3}$	KIDNEYS $x10^{-3}$	LIVER $x10^{-2}$	LUNGS $x10^{-3}$	TESTES $x10^{-2}$
481	281.3	1.23	6.42	2.91	7.57	3.54	4.18	0.92
482	307.3	1.34	6.16	2.82	7.43	3.45	4.16	0.98
483	308.1	0.95	6.10	2.64	7.19	3.27	3.80	0.97
484	301.8	1.34	6.18	2.67	7.47	3.42	4.11	1.01
485	288.9	1.42	6.36	2.99	7.01	3.71	4.41	1.00
486	314.4	1.27	5.99	2.92	7.57	3.64	3.91	0.99
487	297.8	1.15	6.26	2.62	6.97	3.42	4.23	0.92
488	293.3	1.27	6.45	2.50	7.27	3.24	4.33	1.03
489	309.6	1.43	6.10	2.45	6.68	3.59	3.34	0.92
490	315.8	1.02	5.95	2.80	7.81	3.81	4.70	0.95
x	301.83	1.24	6.20	2.73	7.00	3.51	4.16	0.97
std.dev.	11.36	0.16	0.17	0.18	1.04	0.18	0.40	0.04

TITANIUM OXIDE

ANIMAL NUMBER	BODY WT. (GMS.)	ADRENALS $x10^{-4}$	BRAIN $x10^{-3}$	HEART $x10^{-3}$	KIDNEYS $x10^{-3}$	LIVER $x10^{-2}$	LUNGS $x10^{-3}$	TESTES $x10^{-2}$
497	354.70	2.20	5.44	3.07	8.18	4.96	4.31	0.91
498	277.30	2.31	6.67	3.28	7.86	4.08	4.58	1.07
499	293.70	2.32	6.37	3.20	8.04	4.28	4.60	1.04
500	300.40	2.03	6.19	3.20	7.56	4.32	5.23	1.03
501	302.20	1.65	6.12	2.98	7.91	4.04	4.47	0.95
502	290.40	1.76	6.37	3.17	7.58	4.14	4.44	1.03
503	292.30	2.53	6.29	3.11	8.18	4.39	4.24	0.96
504	303.20	1.81	6.56	3.27	7.78	3.78	5.15	1.02
505	290.30	2.07	6.37	3.20	7.82	2.90	4.44	1.01
506	297.40	1.75	6.32	3.26	7.83	4.70	4.64	1.02
x	300.19	2.04	6.27	3.17	7.87	4.16	4.16	1.00
std.dev.	20.59	0.30	0.33	0.10	0.21	0.56	0.33	0.05

DATA ANALYSIS
ORGAN/BODY RATIOS OF MALE FISCHER 344 RATS EXPOSED TO SYNTHETIC GRAPHITE, NATURAL GRAPHITE, AND TITANIUM OXIDE

24 HOURS POST EXPOSURE

	BODY WT.	ADRENALS	BRAIN	HEART	KIDNEY	LIVER	LUNGS	TESTES
BARTLETT'S	*NS*	*NS*	*NS*	*NS*	*NS*	*SIG*	*NS*	*SIG*
ANOVA						*SIG*		*NS*
DUNNETT'S						*NS*		
F-TEST								
KRUSKAL-WALLIS								

---

14 DAY POST EXPOSURE

	BODY WT.	ADRENALS	BRAIN	HEART	KIDNEYS	LIVER	LUNGS	TESTES
BARTLETT'S	*NS*	*NS*	*NS*	*NS*	*SIG*	*SIG*	*NS*	*NS*
ANOVA					*SIG*	*SIG*		
DUNNETT'S					*SIG*	*SIG*		
F-TEST					*NS*	*NS*		
KRUSKAL-WALLIS								

HSHB-MO-B

MEMORANDUM FOR Cdr, US Army Chemical Research, Development and Engineering Center, ATTN: SMCCR-RST-E, Aberdeen Proving Ground, MD 21010-5425

SUBJECT: Exposure of Rat Ears to Stressful Noise Levels

1. In reference to a question from Dr. Sandi Thomson on 10 November concerning noise exposure for rats being used as subject in an inhalation experiment, the following information is provided.

2. The noise in question is a broad band noise with the greatest concentration of energy at 1000 Hz. Measured on a linear scale, the intensity of the noise is 91 decibels (dB) when measured with either a 1/4 inch or 1/2 inch microphone. Of these two microphones, only the 1/4 inch is sensitive at the frequencies which rats hear best (40,000 Hz). The enclosed graph gives the 1/3 octave levels as measured with the 1/4 inch microphone.

3. Based on published research (references 5a and b), it is improbable that the noise levels below 5,000 Hz will cause any stress. Rats are relatively insensitive at these frequencies (reference 5c) and they fail to avoid noise levels as high as 100 dB when the spectrum is biased toward these lower frequencies. Thus, the concentration of energy at 1,000 Hz can be ignored.

4. Rats will, however, actively avoid pulsed noise in the 20,000 to 30,000 Hz range at levels of 96 dB (reference 5d). Since the noise in the inhalation booth is steady rather than pulsed and 22 dB lower than the level found to generate active avoidance, it is reasonable to assume that the noise will not introduce additional stress. In human hearing, a reduction of 10 dB equates to a halving of subjective loudness, and a reduction of 20 dB equates to cutting the loudness to one fourth of its initial value.

5. References.

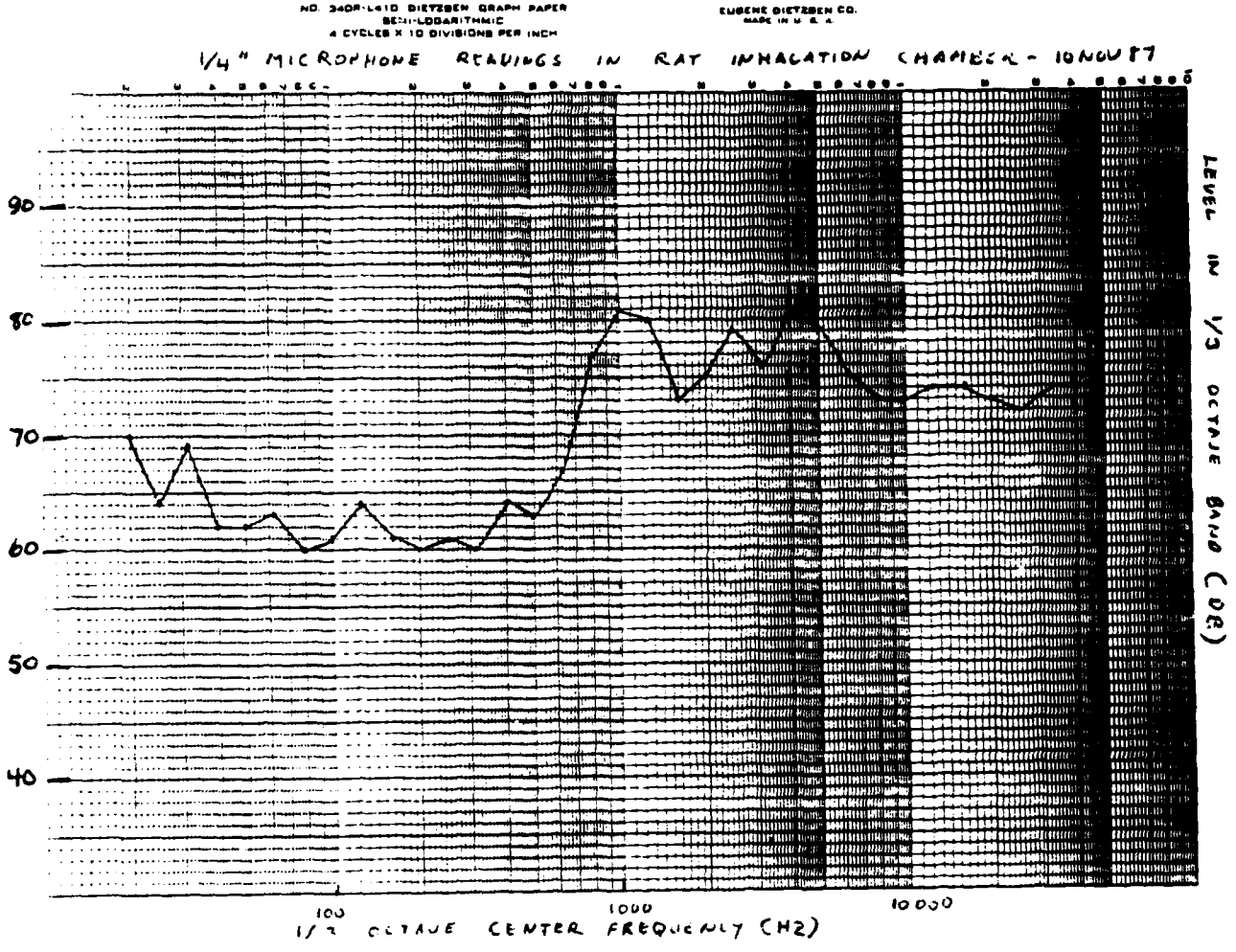
a. Myers, A.K. Contradictory evidence for aversion to auditory stimuli resulting from different test methods. Journal of Comparative and Physiological Psychology. 64: 171-175, 1967.

b. Sprock, C.M., W.E. Howard and F.C. Jacob, Sound as a deterrent to rats and mice. Journal of Wildlife Management. 31: 729-741, 1967.

c. Gourevitch, G. and M.H. Hack, Audibility in the rat, Journal of Comparative and Physiological Psychology. 62: 289-291, 1966.

d. Belluzzi, J.D. and S.P. Grossman, Avoidance learning motivated by high-frequency sound and electric shock. 4: 371-373, 1969.

GEORGE A. LUZ
Program Manager
Environmental Noise


NO. 340R-L410 DIETZGEN GRAPH PAPER
SEMI-LOGARITHMIC
4 CYCLES X 10 DIVISIONS PER INCH
EUGENE DIETZGEN CO.
MADE IN U. S. A.
1/4" MICROPHONE READINGS IN RAT INHALATION CHAMBER - 10NOV87
90
80
70
60
50
40
100
1000
10000
1/3 OCTAVE CENTER FREQUENCY (HZ)
LEVEL IN 1/3 OCTAVE BAND (DB)

Blank

APPENDIX E. AEROSOL CONCENTRATION MEASUREMENTS DURING EXPOSURES AND AUXILIARY CHAMBER MEASUREMENTS

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SUMMARY OF AEROSOL CONCENTRATION MEASUREMENTS DURING EXPOSURES

EXPOSURE DAY #1 13-Dec-87
Protocol #22087000A217

Aerosol Concentration, mg/m^3

Elapsed Time, min.	Chamber #2 Natural Graphite	Chamber #3 Synthetic Graphite	Chamber #4 Titanium Dioxide
16	85.7	124	68.3
42	128	102	80.3
73	112	126	95.8
109	140	100	99.9
133	122	86.7	85.7
163	133	98.8	110
191	122	98.1	100
211	97.3	98.5	96.4
241			92.3

DAY#1 STATISTICS			
Average	117.5	104.3	92.1
Std	18.3	13.6	12.4
Max % Var.	27.1%	20.8%	25.8%
C.V.	15.6%	13.0%	13.5%

Std = Standard deviation

Max % Var. = Maximum % variation of any one measurement from the mean.

C.V. = Coefficient of Variation

AEROSOL CONCENTRATION MEASUREMENTS DURING EXPOSURES

EXPOSURE DAY #2 14-Dec-87
Protocol #22087000A217

Aerosol Concentration, mg/m^3

Elapsed Time, min.	Chamber #2 Natural Graphite	Chamber #3 Synthetic Graphite	Chamber #4 Titanium Dioxide
11		78.2	132
35	74.5	93	135
48	94.8	105	116
60	88.1	95.9	100
86	108	93.9	122
106	101	108	121
136	105	113	91
161	93.8	104	91
180	97.7	103	97.2
208	102	111	92.8
233	89.5	102	115
DAY#2 STATISTICS			
Average	95.4	100.6	110.3
Std	9.8	9.9	16.5
Max % Var.	21.9%	22.3%	22.4%
C.V.	10.3%	9.8%	15.0%

SUMMARY OF AEROSOL CONCENTRATION MEASUREMENTS DURING EXPOSURES

EXPOSURE DAY #3 15-Dec-87
Protocol #22087000A217

Aerosol Concentration, mg/m^3

Elapsed Time, min.	Chamber #2 Natural Graphite	Chamber #3 Synthetic Graphite	Chamber #4 Titanium Dioxide
13	99.5	92.1	103
25	102	90.5	72.1
43			92.5
57	82	83.5	95.9
86	93.2	89.5	88.6
112	106	106	73.5
132			118
152	110	113	109
179	102	90	117
208	75.7	115	120
232	105	103	113
DAY#3 STATISTICS			
Average	97.3	98.1	100.2
Std	11.5	11.4	17.2
Max % Var.	22.2%	17.2%	28.0%
C.V.	11.8%	11.6%	17.2%

Std = Standard deviation

Max % Var. = Maximum % variation of any one measurement from the mean.

C.V. = Coefficient of Variation

AEROSOL CONCENTRATION MEASUREMENTS DURING EXPOSURES

EXPOSURE DAY #4 16-Dec-87
Protocol #22087000A217

Aerosol Concentration, mg/m^3

Elapsed Time, min.	Chamber #2 Natural Graphite	Chamber #3 Synthetic Graphite	Chamber #4 Titanium Dioxide
11	102	89.3	108
31	96.9	96.3	115
57	108	94.9	116
86	94.3	105	122
116	114	93	93.6
144	82.5	101	90.3
169	99.8	92.1	85.5
198	117	121	81.7
236	91.2	97.7	103
DAY#4 STATISTICS			
Average	100.6	98.9	101.7
Std	11	9.5	14.5
Max % Var.	18.0%	22.3%	20.0%
C.V.	10.9%	9.6%	14.3%

SUMMARY OF AEROSOL CONCENTRATION MEASUREMENTS DURING EXPOSURES

Protocol #22087000A217
OVERALL EXPOSURE STATISTICS

Aerosol Concentration, mg/m^3

Measure	Chamber #2 Natural Graphite	Chamber #3 Synthetic Graphite	Chamber #4 Titanium Dioxide
Average	102.1	100.4	101.5
Std	14.9	10.8	16.2
S.E.	7.45	5.4	8.1
Max % Var.	22.3%	20.7%	24.1%
C.V.	14.6%	10.8%	16.0%

Std = Standard deviation

S.E. = Standard Error

Max % Var. = Average of the maximum percent variation from each exposure day.

C.V. = Coefficient of Variation

Kruskal-Wallis Test: Input Data - Chamber Concentration, mg/cubic meter

Dispersed Material

Natural Graphite	Synthetic Graphite	Titanium Dioxide
89.5	100	88.6
106	105	100
140	93.9	116
105	106	93.6
74.5	94.9	116
105	89.5	95.9
128	95.9	117
102	89.3	97.2
122	96.3	68.3
102	108	108
82.5	93	118
102	92.1	110
114	92.1	120
91.2	97.7	91
112	102	99.9
102	98.1	91
88.1	113	121
101	98.8	113
108	102	122
99.8	124	115
133	111	122
99.5	83.5	90.3
122	90.5	81.7
97.7	113	96.4
117	86.7	80.3
97.3	90	109
110	93	92.3
96.9	98.5	85.7
94.3	103	92.5
93.2	121	85.5
82	103	92.8
94.8	101	95.8
108	104	132
85.7	126	103
75.7	78.2	73.5
93.8	115	115
	105	100
n = 36		103
	n = 37	135
		72.1
		n = 40

Kruskal-Wallis Test: Ranks

Dispersed Material		
Natural Graphite	Synthetic Graphite	Titanium Dioxide
19.5	58	17
77.5	74.5	58
113	37	95.5
74.5	77.5	35
4	40	95.5
74.5	19.5	42.5
109	42.5	97.5
64.5	18	47
104.5	44	1
64.5	80.5	80.5
10	32.5	99
64.5	27.5	84.5
91	27.5	100
26	49.5	24.5
87	64.5	56
64.5	51	24.5
16	89	101.5
60.5	53	89
80.5	64.5	104.5
55	107	93
111	86	104.5
54	11	22
104.5	23	8
49.5	89	45
97.5	15	7
48	21	83
84.5	32.5	29
46	52	13.5
38	69.5	30
34	101.5	12
9	69.5	31
39	60.5	41
80.5	72	110
13.5	108	69.5
5	6	3
36	93	93
	74.5	58
		69.5
		112
		2
Sum = 2110.5	Sum = 2041.5	Sum = 2289

Kruskal-Wallis Test: Summary

Null Hypothesis: Aerosol Concentrations for each treatment are the same.

Treatment:	Natural Graphite	Synthetic Graphite	Titanium Dioxide
Rank Sum (RS):	2110.5	2041.5	2289
Number (n):	36	37	40
RS^2 / n =	123728.0	112641.1	130988.0

H Value = 0.205150

Degrees of Freedom = 2

Critical Chi-Square Value @ 0.05 significance Level = 5.991

H < 5.991, therefore, accept null hypothesis.

Auxiliary Chamber Measurements

Temperature, degrees F *

Chamber	Day #1	Day #2	Day #3	Day #4
#1 (Control)	72.8	73.3	73.8	73.3
#2 (NG)	73.9	74.1	73.9	74.3
#3 (SG)	72.9	73.2	73.0	72.8
#4 (TiO_2)	71.8	72.0	72.2	71.9

* All values are the average of four measurements with a standard deviation of +/- 1 deg. F or less

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Relative Humidity, percent **

Chamber	Day #1	Day #2	Day #3	Day #4
1# (Control)	29.0	29.6	34.0	30.2

** All values are the average of four measurements with a standard deviation of +/- 1 percent or less.

Chamber Flow Data

Chamber	Flowrate, lpm ^	Air Exchanges/hr ^^
#1 (Control)	607	36.4
#2 (NG)	1182	70.9
#3 (SG)	1400	84.0
#4 (TiO_2)	1051	63.1

^ Average value computed from four hot wire measurements with a coefficient of variation of 12 percent or less.

^^ Based on a chamber volume of 1000 liters.

Blank

APPENDIX F. PATHOLOGY REPORTS

Blank

PAI Pathology Associates, Inc.

10075 Tyler Place
Hyatt Park II
Ijamsville, Maryland 21754
(301) 663-1644

PATHOLOGY REPORT
FOR

COMPARATIVE ACUTE INHALATION SCREEN OF IRON OXIDE AND GRAPHITE DUSTS FOUR DAY EXPOSURE WITH ONE DAY RECOVERY

PROTOCOL NO. 22087000A217

CONTRACT NO. DAAA15-85-D-0002

TABLE OF CONTENTS

I. PATHOLOGY REPORT

PATHOLOGY REPORT

COMPARATIVE ACUTE INHALATION SCREEN OF IRON OXIDE AND GRAPHITE DUSTS FOUR DAY EXPOSURE WITH ONE DAY RECOVERY

INTRODUCTION AND METHODS

Male F344 rats were divided into four groups of twenty each, and three of the groups were exposed to 100 mg/m^3 of natural graphite (Micro 650), synthetic graphite (Micro 260), or titanium dioxide (substituted for iron oxide) on four consecutive days, four hours/day. A control group of twenty rats was similarly exposed to air. Following the repeated exposure, ten rats per group were allowed to recover for one day after which time they were killed by carbon dioxide asphyxiation and necropsied. The remaining rats were allowed to recover for 14 days after the final exposure before they were killed and necropsied. The pathology findings in the 14-day recovery rats will be presented in a later pathology report. At necropsy, the total body weight and the organ weights of adrenals, brain, heart, kidneys, liver, lung, and testes were recorded.

All organs and tissues required by contract were processed through paraffin, sectioned at approximately 6 µm, stained with hematoxylin and eosin, and examined microscopically. In addition to the required tissues, an attempt was made to evaluate peribronchial lymph nodes as well. An occasional tissue was lost during necropsy or processing and could not be examined microscopically.

RESULTS AND DISCUSSION

Gross Findings

The lungs of 8/10 rats in the group exposed to natural graphite and 4/10 rats exposed to synthetic graphite were discolored (dark or black) as shown in Section II. The remaining gross observations were miscellaneous, non-treatment-related changes. No apparent differences in body or organ weights were noted between groups (Section III).

Gross lesions noted at necropsy or during trimming have been correlated with microscopic diagnoses, where possible, and are listed by animal in the CORRELATION OF GROSS AND MICROSCOPIC FINDINGS TABLE (Section VI). Correlative microscopic findings could not be determined for all gross lesions.

Microscopic Findings

Summary data are presented in the PROJECT SUMMARY TABLES (Section IV), which lists by group the number of animals having a given lesion. Microscopic findings

for individual animals are presented by group in the TABULATED ANIMAL DATA TABLES (Section V). The codes used in the entries in these tables are explained in the Reports Code Table (Appendix 1), while topographic/morphologic abbreviations are explained in the Abbreviations List (Appendix 2).

Treatment-related changes were apparent in the lungs of all rats other than the controls. In each case, brown to black, isotropic pigment was present either free or within macrophages in terminal airways and alveoli. Microscopically, the three types of pigment were indistinguishable from each other. No other changes in the lungs were associated with the pigment. Focal hemorrhage was present in at least one rat from each group, to include the controls, and is believed to be associated with the carbon dioxide asphyxiation. No pigment was present in the peribronchial lymph nodes.

The severity of the pigmentation was similar within and between groups. It was graded as minimal or mild in all of the exposed rats with only 1/10, 0/10, and 3/10 rats receiving minimal grades in the synthetic graphite, natural graphite, and titanium dioxide groups, respectively. The reason why the pigmentation could not be seen grossly in all exposed rats is not known.

Similar pigment was seen in the colon of two rats exposed to natural graphite and was probably due to grooming or swallowing of material removed from the upper or lower respiratory tract by the mucociliary apparatus.

Several lesions commonly found in rats such as cardiomyopathy and nephropathy were present in rats in more than one dose group. In addition, thymic hemorrhage which is probably associated with euthanasia was present in several animals. These findings and all other lesions not mentioned are incidental findings and should not be associated with the test substances in any way.

CONCLUSIONS

Exposure of male F344 rats to natural graphite, synthetic graphite, or titanium dioxide by chamber inhalation for four days, four hours/day, followed by a one day recovery resulted in lung changes in all exposed rats consisting of pigmentation (particulate deposition) in alveoli and terminal airways. Pigment was both free and within macrophages. No significant differences in severity or composition of the pigment deposition between groups could be determined.

Lucas H. Brennecke, D.V.M.
Diplomate, ACVP
March 24, 1988

APPENDIX I:
REPORTS CODE TABLE

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
OF IRON OXIDE AND GRAPHITE DUSTS
24 HOUR POST-EXPOSURE SACRIFICE

Reports Code Table

N	Tissues within normal histological limits
A	Autolysis precluding adequate evaluation
P	Paired organ missing
U	Tissues unavailable/unsuitable for evaluation
S	Tissues not applicable to animal
*	Tissues not examined/not required by protocol

1	minimal
2	mild
3	moderate
4	marked
)	focal
]	diffuse
>	multifocal
P	Present
B	Neoplasm, Benign
M	Neoplasm, Malignant without Metastasis
C	Neoplasm, Malignant with Metastasis
X	Metastatic Site (+)
-	No data entered

(End of Report)

APPENDIX II:
ABBREVIATIONS LIST

ABBREVIATIONS LIST

EPITH	Epithelium
INFLAM	Inflammation
OLF	Olfactory
RESP	Respiratory

II. GROSS NECROPSY FINDINGS

TABLE II-1

GROSS NECROPSY FINDINGS

Dose Group	Animal No.	Tissue - Lesion
Control		
	421	Kidney - Foci
Synthetic graphite		
	452	Lung - Mottled
	453	Pancreas - Nodule
	456	Thymus - Foci
	458	Lung - Grey
Natural graphite		
	481	Lung - Grey
	482	Lung - Mottled
	483	Lung - Foci
	487	Lung - Dark
		Bronchial lymph node - Enlarged
	489	Lung - Foci
	490	Lung - Grey
		Mesentery - Nodule
Titanium dioxide		
	518	Lung - Foci

III. ORGAN AND BODY WEIGHTS

Table III-1
Organ and Body Weights (grams)

Males

Animal No.	Group	Body Wt.	Adrenals	Brain	Heart	Kidney	Liver	Lungs	Testes
400	Control	280.4	.058	1.83	.90	2.37	11.59	1.23	2.98
401	Control	288.4	.065	1.79	.93	2.48	12.61	1.17	2.97
402	Control	279.8	.051	1.86	.96	2.33	11.44	1.24	2.94
403	Control	292.2	.075	1.80	.98	2.48	12.75	1.12	2.82
404	Control	279.0	.042	1.84	.91	2.34	11.98	1 18	2.87
405	Control	306.5	.064	1.96	1.04	2.75	13.01	1.30	3.33
406	Control	295.9	.068	1.92	.96	2.44	12.83	1.46	2.83
407	Control	284.3	.054	1.87	.95	2.23	11.57	1.26	3.01
408	Control	278.8	.061	1.84	.91	2.22	11.43	1.21	2.66
409	Control	298.1	.062	1.87	.98	2.54	14.65	1.07	2.90
Mean:		288.3	.060	1.86	.95	2.42	12.39	1.22	2.93
433	260	272.0	.051	1.77	.81	2.25	12.58	1.16	2.66
434	260	294.3	.064	1.91	.96	2.37	12.45	1.44	3.00
435	260	286.6	.058	1.90	.90	2.27	11.46	1.45	2.88
436	260	284.9	.056	1.88	.91	2.31	10.70	1.29	2.91
437	260	266.1	.049	1.80	.91	2.11	10.03	1.25	3.05
438	260	284.5	.052	1.83	.87	2.33	12.66	1.35	2.82
439	260	275.4	.066	1.87	.91	2.31	13.29	1.34	2.98
440	260	273.2	.049	1.64	.92	2.15	11.02	1.36	2.71
441	260	305.1	.062	1.89	1.03	2.46	12.66	1.32	3.01
442	260	296.3	.060	1.89	1.01	2.47	14.96	1.36	3.06
Mean:		283.8	.057	1.84	.92	2.30	12.18	1.33	2.91
465	650	281.7	.071	1.88	.98	2.33	11.60	1.34	2.87
466	650	304.7	.065	1.89	.93	2.36	12.58	1.69	3.15
467	650	284.2	.056	1.82	.92	2.30	11.04	1.35	2.91
468	650	293.8	.057	1.89	.98	2.37	12.22	1.46	3.03
469	650	297.0	.049	1.90	.93	2.43	12.47	1.37	3.11
470	650	296.4	.060	1.87	.98	2.36	13.13	1.34	3.08
471	650	273.7	.061	1.83	.90	2.06	10.58	1.51	3.16
472	650	301.3	.050	1.86	.97	2.33	12.29	1.51	3.17
473	650	290.3	.062	1.90	.88	2.35	11.92	1.26	3.19
474	650	304.5	.062	1.83	.95	2.40	13.38	1.58	3.04
Mean:		292.8	.059	1.87	.94	2.33	12.12	1.44	3.07

Animal No.	Group	Body Wt.	Adrenals	Brain	Heart	Kidney	Liver	Lungs	Testes
497	Ti.Diox.	354.7	.078	1.93	1.09	2.90	17.58	1.53	3.22
498	Ti.Diox.	277.3	.064	1.85	.91	2.18	11.30	1.27	2.98
499	Ti.Diox.	293.7	.068	1.87	.94	2.36	12.58	1.35	3.04
500	Ti.Diox.	300.4	.061	1.86	.96	2.27	12.99	1.57	3.10
501	Ti.Diox.	302.2	.052	1.85	.90	2.39	12.22	1.35	2.87
502	Ti.Diox.	290.4	.051	1.85	.92	2.20	12.01	1.29	3.11
503	Ti.Diox.	292.3	.074	1.84	.91	2.39	12.83	1.24	2.81
504	Ti.Diox.	303.2	.055	1.99	.99	2.36	11.47	1.56	3.09
505	Ti.Diox.	290.3	.060	1.85	.93	2.27	13.38	1.29	2.92
506	Ti.Diox.	297.4	.052	1.88	.97	2.33	13.98	1.38	3.02
Mean:		300.2	.062	1.88	.95	2.37	13.03	1.38	3.02

IV. SUMMARY TABLE OF MICROSCOPIC FINDINGS BY GROUP

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
OF IRON OXIDE AND GRAPHITE DUSTS
24 HOUR POST-EXPOSURE SACRIFICE

Project Summary Table

SUMMARY: Incidence of NEOPLASTIC and NON-NEOPLASTIC Microscopic Findings

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PROJECT ID. NO: G-TI02 FATES: ALL
PAGE 1 DAYS: ALL SEX: MALE

GROUP:		CONTROL	MICRO260	MICRO650	TI02
NUMBER OF ANIMALS:		10	10	10	10
		#	#	#	#
BRAIN	# Ex	10	10	10	10
HEMORRHAGE		0	1	0	0
SCIATIC NERVE	# Ex	10	9	10	10
SPINAL CORD	# Ex	10	10	10	10
ZYMBAL'S GLAND	# Ex	10	10	9	9
SALIVARY GLAND	# Ex	10	10	10	10
PANCREAS	# Ex	10	10	10	10
MANDIBULAR LYMPH NODE	# Ex	10	10	10	10
THYMUS	# Ex	10	10	10	10
HEMORRHAGE		0	2	3	1
TRACHEA	# Ex	10	10	10	10
LARYNX	# Ex	10	10	10	10
THYROIDS	# Ex	9	10	10	10
PARATHYROIDS	# Ex	9	8	9	9
PITUITARY	# Ex	10	10	10	10

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
OF IRON OXIDE AND GRAPHITE DUSTS
24 HOUR POST-EXPOSURE SACRIFICE

Project Summary Table

SUMMARY: Incidence of NEOPLASTIC and NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: G-TI02 FATES: ALL
PAGE 2 DAYS: ALL SEX: MALE

GROUP:		CONTROL	MICRO260	MICRO650	TiO2
NUMBER OF ANIMALS:		10	10	10	10
		#	#	#	#
LUNGS	# Ex	10	10	10	10
HEMORRHAGE		1	3	1	2
PIGMENTATION		0	10	10	10
ARTERY-MINERALIZATION		0	0	1	0
INTERSTITIUM-INFLAMMATION		2	0	1	0
BRONCHIAL LYMPH NODE	# Ex	3	5	10	7
EYE	# Ex	10	10	10	10
SCLERA-MINERALIZATION		0	0	1	2
SCLERA-INFLAMMATION, ACUTE		0	0	0	1
CORNEA-DEGENERATION		0	0	0	1
FORESTOMACH	# Ex	10	10	10	10
GLANDULAR STOMACH	# Ex	10	10	10	10
ESOPHAGUS	# Ex	10	10	10	10
DUODENUM	# Ex	10	10	10	10
COLON	# Ex	10	10	10	10
PIGMENTATION		0	0	2	0
MESENTERIC LYMPH NODE	# Ex	10	10	10	10
LIVER	# Ex	10	10	10	10
HEPATODIAPHRAGMATIC NODULE		1	1	0	1
SPLEEN	# Ex	10	10	10	10

Project Summary Table

SUMMARY: Incidence of NEOPLASTIC and NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: G-TI02 FATES: ALL
PAGE 3 DAYS: ALL SEX: MALE

GROUP:		CONTROL	MICRO260	MICRO650	TiO2
NUMBER OF ANIMALS:		10	10	10	10
		#	#	#	#
HEART	# Ex	10	10	10	10
CARDIOMYOPATHY		5	1	1	3
KIDNEYS	# Ex	10	10	10	10
NEPHROPATHY		1	2	3	3
SKELETAL MUSCLE	# Ex	10	10	10	10
ADRENAL CORTEX	# Ex	10	10	10	10
ADRENAL MEDULLA	# Ex	10	10	10	10
TESTES	# Ex	10	10	10	10
SEMINAL VESICLES	# Ex	10	10	10	10
SKIN	# Ex	10	10	10	10
URINARY BLADDER	# Ex	10	10	10	10
MINERALIZATION		1	0	0	0
PROSTATE	# Ex	10	10	10	10
BONE (STERNUM)	# Ex	10	10	10	10
BONE MARROW	# Ex	10	10	10	10
NOSE	# Ex	10	10	10	10
OLF EPITH-INFLAM, ACUTE		0	1	0	0

Project Summary Table

SUMMARY: Incidence of NEOPLASTIC and NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: G-TI02 FATES: ALL
PAGE 4 DAYS: ALL SEX: MALE

GROUP:		CONTROL	MICR0260	MICR0650	Ti02
NUMBER OF ANIMALS:		10	10	10	10
		#	#	#	#
NOSE	# Ex	10	10	10	10
RESP EPITH-HYPERPLASIA		0	0	2	0

(End of Report)

V. TABULATED INDIVIDUAL ANIMAL TABLE

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
OF IRON OXIDE AND GRAPHITE DUSTS
24 HOUR POST-EXPOSURE SACRIFICE

Tabulated Animal Data

PROJECT ID: G-TI02 GROUP: CONTROL SEX: M DAYS: ALL
PAGE 1 FATES: SCHEDULED SACRIFICE

ANIMAL ID. NO:	400	401	402	403	404	405	406	407	408	409
BRAIN	N	N	N	N	N	N	N	N	N	N
SCIATIC NERVE	N	N	N	N	N	N	N	N	N	N
SPINAL CORD	N	N	N	N	N	N	N	N	N	N
ZYMBAL'S GLAND	N	N	N	N	N	N	N	N	N	N
SALIVARY GLAND	N	N	N	N	N	N	N	N	N	N
PANCREAS	N	N	N	N	N	N	N	N	N	N
MANDIBULAR LYMPH NODE	N	N	N	N	N	N	N	N	N	N
THYMUS	N	N	N	N	N	N	N	N	N	N
TRACHEA	N	N	N	N	N	N	N	N	N	N
LARYNX	N	N	N	N	N	N	N	N	N	N
THYROIDS	U	N	N	N	N	N	N	N	N	N
PARATHYROIDS	U	N	N	N	N	N	N	N	N	N

(Report Continued)

Tabulated Animal Data

PROJECT ID: G-TI02 GROUP: CONTROL SEX: M DAYS: ALL
PAGE 2 FATES: SCHEDULED SACRIFICE

ANIMAL ID. NO:	400	401	402	403	404	405	406	407	408	409
PITUITARY	N	N	N	N	N	N	N	N	N	N
LUNGS		N	N			N	N	N	N	N
HEMORRHAGE	(1)	-	-	-	-	-	-	-	-	-
INTERSTITIUM-INFLAMMATION	-	-	-	(1)	(1)	-	-	-	-	-
BRONCHIAL LYMPH NODE	N	N	N	U	U	U	U	U	U	U
EYE	N	N	N	N	N	N	N	N	N	N
FORESTOMACH	N	N	N	N	N	N	N	N	N	N
GLANDULAR STOMACH	N	N	N	N	N	N	N	N	N	N
ESOPHAGUS	N	N	N	N	N	N	N	N	N	N
DUODENUM	N	N	N	N	N	N	N	N	N	N
COLON	N	N	N	N	N	N	N	N	N	N
MESENTERIC LYMPH NODE	N	N	N	N	N	N	N	N	N	N
LIVER	N	N	N	N	N		N	N	N	N
HEPATODIAPHRAGMATIC NODULE	-	-	-	-	-	P	-	-	-	-

(Report Continued)

Tabulated Animal Data

PROJECT ID: G-TI02 GROUP: CONTROL SEX: M DAYS: ALL
PAGE 3 FATES: SCHEDULED SACRIFICE

ANIMAL ID. NO:	400	401	402	403	404	405	406	407	408	409
SPLEEN	N	N	N	N	N	N	N	N	N	N
HEART	N						N	N	N	N
CARDIOMYOPATHY	-	1	1	2	1	1	-	-	-	-
KIDNEYS	N	N	N	N	N	N	N		N	N
NEPHROPATHY	-	-	-	-	-	-	-	1	-	-
SKELETAL MUSCLE	N	N	N	N	N	N	N	N	N	N
ADRENAL CORTEX	N	N	N	N	N	N	N	N	N	N
ADRENAL MEDULLA	N	N	N	N	N	N	N	N	N	N
TESTES	N	N	N	N	N	N	N	N	N	N
SEMINAL VESICLES	N	N	N	N	N	N	N	N	N	N
SKIN	N	N	N	N	N	N	N	N	N	N
URINARY BLADDER	N	N		N	N	N	N	N	N	N
MINERALIZATION	-	-	(1)	-	-	-	-	-	-	-
PROSTATE	N	N	N	N	N	N	N	N	N	N

(Report Continued)

Tabulated Animal Data

PROJECT ID: G-TI02 GROUP: CONTROL SEX: M DAYS: ALL
PAGE 4 FATES: SCHEDULED SACRIFICE

ANIMAL ID. NO:	400	401	402	403	404	405	406	407	408	409
BONE (STERNUM)	N	N	N	N	N	N	N	N	N	N
BONE MARROW	N	N	N	N	N	N	N	N	N	N
NOSE	N	N	N	N	N	N	N	N	N	N

(Report Continued)

Tabulated Animal Data

PROJECT ID: G-TI02 GROUP: MICRO260 SEX: M DAYS: ALL
PAGE 5 FATES: SCHEDULED SACRIFICE

ANIMAL ID. NO:	433	434	435	436	437	438	439	440	441	442
BRAIN	N	N	N	N	N	N	N	N	N	
HEMORRHAGE	-	-	-	-	-	-	-	-	-	(1)
SCIATIC NERVE	N	U	N	N	N	N	N	N	N	N
SPINAL CORD	N	N	N	N	N	N	N	N	N	N
ZYMBAL'S GLAND	N	N	N	N	N	N	N	N	N	N
SALIVARY GLAND	N	N	N	N	N	N	N	N	N	N
PANCREAS	N	N	N	N	N	N	N	N	N	N
MANDIBULAR LYMPH NODE	N	N	N	N	N	N	N	N	N	N
THYMUS	N	N		N		N	N	N	N	N
HEMORRHAGE	-	-	1	-	1	-	-	-	-	-
TRACHEA	N	N	N	N	N	N	N	N	N	N
LARYNX	N	N	N	N	N	N	N	N	N	N
THYROIDS	N	N	N	N	N	N	N	N	N	N

(Report Continued)

Tabulated Animal Data

PROJECT ID: G-TI02 GROUP: MICRO260 SEX: M DAYS: ALL
PAGE 6 FATES: SCHEDULED SACRIFICE

ANIMAL ID. NO:	433	434	435	436	437	438	439	440	441	442
PARATHYROIDS	N	N	N	U	N	U	N	N	N	N
PITUITARY	N	N	N	N	N	N	N	N	N	N
LUNGS										
HEMORRHAGE	(1)	(1)	-	-	-	-	-	(1)	-	-
PIGMENTATION	2	2	1	2	2	2	2	2	2	2
BRONCHIAL LYMPH NODE	U	N	U	N	N	U	U	N	U	N
EYE	N	N	N	N	N	N	N	N	N	N
FORESTOMACH	N	N	N	N	N	N	N	N	N	N
GLANDULAR STOMACH	N	N	N	N	N	N	N	N	N	N
ESOPHAGUS	N	N	N	N	N	N	N	N	N	N
DUODENUM	N	N	N	N	N	N	N	N	N	N
COLON	N	N	N	N	N	N	N	N	N	N
MESENTERIC LYMPH NODE	N	N	N	N	N	N	N	N	N	N

(Report Continued)

Tabulated Animal Data

PROJECT ID: G-TI02 GROUP: MICRO260 SEX: M DAYS: ALL
PAGE 7 FATES: SCHEDULED SACRIFICE

ANIMAL ID. NO:	433	434	435	436	437	438	439	440	441	442
LIVER	N	N	N	N		N	N	N	N	N
HEPATODIAPHRAGMATIC NODULE	-	-	-	-	P	-	-	-	-	-
SPLEEN	N	N	N	N	N	N	N	N	N	N
HEART	N	N	N	N	N		N	N	N	N
CARDIOMYOPATHY	-	-	-	-	-	1	-	-	-	-
KIDNEYS		N	N	N	N		N	N	N	N
NEPHROPATHY	1	-	-	-	-	1	-	-	-	-
SKELETAL MUSCLE	N	N	N	N	N	N	N	N	N	N
ADRENAL CORTEX	N	N	N	N	N	N	N	N	N	N
ADRENAL MEDULLA	N	N	N	N	N	N	N	N	N	N
TESTES	N	N	N	N	N	N	N	N	N	N
SEMINAL VESICLES	N	N	N	N	N	N	N	N	N	N
SKIN	N	N	N	N	N	N	N	N	N	N
URINARY BLADDER	N	N	N	N	N	N	N	N	N	N

(Report Continued)

Tabulated Animal Data

PROJECT ID: G-TI02 GROUP: MICRO260 SEX: M DAYS: ALL
PAGE 8 FATES: SCHEDULED SACRIFICE

ANIMAL ID. NO:	433	434	435	436	437	438	439	440	441	442
PROSTATE	N	N	N	N	N	N	N	N	N	N
BONE (STERNUM)	N	N	N	N	N	N	N	N	N	N
BONE MARROW	N	N	N	N	N	N	N	N	N	N
NOSE	N	N	N	N	N	N	N	N	N	
OLF EPITH-INFLAM, ACUTE	-	-	-	-	-	-	-	-	-	(1)

(Report Continued)

Tabulated Animal Data

PROJECT ID: G-TI02 GROUP: MICR0650 SEX: M DAYS: ALL
PAGE 9 FATES: SCHEDULED SACRIFICE

ANIMAL ID. NO:	465	466	467	468	469	470	471	472	473	474
BRAIN	N	N	N	N	N	N	N	N	N	N
SCIATIC NERVE	N	N	N	N	N	N	N	N	N	N
SPINAL CORD	N	N	N	N	N	N	N	N	N	N
ZYMBAL'S GLAND	N	N	N	N	N	N	N	N	U	N
SALIVARY GLAND	N	N	N	N	N	N	N	N	N	N
PANCREAS	N	N	N	N	N	N	N	N	N	N
MANDIBULAR LYMPH NODE	N	N	N	N	N	N	N	N	N	N
THYMUS	N			N	N	N	N		N	N
HEMORRHAGE	-	2	1	-	-	-	-	2	-	-
TRACHEA	N	N	N	N	N	N	N	N	N	N
LARYNX	N	N	N	N	N	N	N	N	N	N
THYROIDS	N	N	N	N	N	N	N	N	N	N

(Report Continued)

Tabulated Animal Data

PROJECT ID: G-TI02 GROUP: MICR0650 SEX: M DAYS: ALL
PAGE 10 FATES: SCHEDULED SACRIFICE

ANIMAL ID. NO:	465	466	467	468	469	470	471	472	473	474
PARATHYROIDS	U	N	N	N	N	N	N	N	N	N
PITUITARY	N	N	N	N	N	N	N	N	N	N
LUNGS										
HEMORRHAGE	-	-	-	-	(1)	-	-	-	-	-
PIGMENTATION	2	2	2	2	2	2	2	2	2	2
ARTERY-MINERALIZATION	-	-	-	-	-	(1)	-	-	-	-
INTERSTITIUM-INFLAMMATION	-	-	-	(2)	-	-	-	-	-	-
BRONCHIAL LYMPH NODE	N	N	N	N	N	N	N	N	N	N
EYE	N		N	N	N	N	N	N	N	N
SCLERA-MINERALIZATION	-	(2)	-	-	-	-	-	-	-	-
FORESTOMACH	N	N	N	N	N	N	N	N	N	N
GLANDULAR STOMACH	N	N	N	N	N	N	N	N	N	N
ESOPHAGUS	N	N	N	N	N	N	N	N	N	N
DUODENUM	N	N	N	N	N	N	N	N	N	N
COLON	N	N	N	N	N		N	N	N	
PIGMENTATION	-	-	-	-	-	1	-	-	-	1

(Report Continued)

Tabulated Animal Data

PROJECT ID: G-TI02 GROUP: MICRO650 SEX: M DAYS: ALL
PAGE 11 FATES: SCHEDULED SACRIFICE

ANIMAL ID. NO:	465	466	467	468	469	470	471	472	473	474
MESENTERIC LYMPH NODE	N	N	N	N	N	N	N	N	N	N
LIVER	N	N	N	N	N	N	N	N	N	N
SPLEEN	N	N	N	N	N	N	N	N	N	N
HEART	N	N	N	N	N		N	N	N	N
CARDIOMYOPATHY	-	-	-	-	-	1	-	-	-	-
KIDNEYS	N	N	N		N	N	N	N		
NEPHROPATHY	-	-	-	1	-	-	-	-	1	1
SKELETAL MUSCLE	N	N	N	N	N	N	N	N	N	N
ADRENAL CORTEX	N	N	N	N	N	N	N	N	N	N
ADRENAL MEDULLA	N	N	N	N	N	N	N	N	N	N
TESTES	N	N	N	N	N	N	N	N	N	N
SEMINAL VESICLES	N	N	N	N	N	N	N	N	N	N
SKIN	N	N	N	N	N	N	N	N	N	N

(Report Continued)

Tabulated Animal Data

PROJECT ID: G-TI02 GROUP: MICRO650 SEX: M DAYS: ALL
PAGE 12 FATES: SCHEDULED SACRIFICE

ANIMAL ID. NO:	465	466	467	468	469	470	471	472	473	474
URINARY BLADDER	N	N	N	N	N	N	N	N	N	N
PROSTATE	N	N	N	N	N	N	N	N	N	N
BONE (STERNUM)	N	N	N	N	N	N	N	N	N	N
BONE MARROW	N	N	N	N	N	N	N	N	N	N
NOSE	N	N	N	N	N	N	N			N
RESP EPITH-HYPERPLASIA	-	-	-	-	-	-	-	(1)	(1)	-

(Report Continued)

Tabulated Animal Data

PROJECT ID: G-TI02 GROUP: Ti02 SEX: M DAYS: ALL
PAGE 13 FATES: SCHEDULED SACRIFICE

ANIMAL ID. NO:	497	498	499	500	501	502	503	504	505	506
BRAIN	N	N	N	N	N	N	N	N	N	N
SCIATIC NERVE	N	N	N	N	N	N	N	N	N	N
SPINAL CORD	N	N	N	N	N	N	N	N	N	N
ZYMBAL'S GLAND	N	N	U	N	N	N	N	N	N	N
SALIVARY GLAND	N	N	N	N	N	N	N	N	N	N
PANCREAS	N	N	N	N	N	N	N	N	N	N
MANDIBULAR LYMPH NODE	N	N	N	N	N	N	N	N	N	N
THYMUS	N	N	N	N	N	N	N	N		N
HEMORRHAGE	-	-	-	-	-	-	-	-	1	-
TRACHEA	N	N	N	N	N	N	N	N	N	N
LARYNX	N	N	N	N	N	N	N	N	N	N
THYROIDS	N	N	N	N	N	N	N	N	N	N

(Report Continued)

Tabulated Animal Data

PROJECT ID: G-TI02 GROUP: TI02 SEX: M DAYS: ALL
PAGE 14 FATES: SCHEDULED SACRIFICE

ANIMAL ID. NO:	497	498	499	500	501	502	503	504	505	506
PARATHYROIDS	N	N	N	N	N	U	N	N	N	N
PITUITARY	N	N	N	N	N	N	N	N	N	N
LUNGS										
HEMORRHAGE	-	-	-	(2)	-	(1)	-	-	-	-
PIGMENTATION	2	1	1	2	2	2	2	1	2	2
BRONCHIAL LYMPH NODE	N	U	N	N	U	U	N	N	N	N
EYE	N	N	N	N		N	N		N	
SCLERA-MINERALIZATION	-	-	-	-	(1)	-	-	-	-	(2)
SCLERA-INFLAMMATION, ACUTE	-	-	-	-	(2)	-	-	-	-	-
CORNEA-DEGENERATION	-	-	-	-	-	-	-	(1)	-	-
FORESTOMACH	N	N	N	N	N	N	N	N	N	N
GLANDULAR STOMACH	N	N	N	N	N	N	N	N	N	N
ESOPHAGUS	N	N	N	N	N	N	N	N	N	N
DUODENUM	N	N	N	N	N	N	N	N	N	N
COLON	N	N	N	N	N	N	N	N	N	N

(Report Continued)

Tabulated Animal Data

PROJECT ID: G-TI02 GROUP: Ti02 SEX: M DAYS: ALL
PAGE 15 FATES: SCHEDULED SACRIFICE

ANIMAL ID. NO:	497	498	499	500	501	502	503	504	505	506
MESENTERIC LYMPH NODE	N	N	N	N	N	N	N	N	N	N
LIVER		N	N	N	N	N	N	N	N	N
HEPATODIAPHRAGMATIC NODULE	P	-	-	-	-	-	-	-	-	-
SPLEEN	N	N	N	N	N	N	N	N	N	N
HEART	N			N	N	N	N	N		N
CARDIOMYOPATHY	-	1	1	-	-	-	-	-	1	-
KIDNEYS			N	N	N	N	N	N		N
NEPHROPATHY	1	1	-	-	-	-	-	-	1	-
SKELETAL MUSCLE	N	N	N	N	N	N	N	N	N	N
ADRENAL CORTEX	N	N	N	N	N	N	N	N	N	N
ADRENAL MEDULLA	N	N	N	N	N	N	N	N	N	N
TESTES	N	N	N	N	N	N	N	N	N	N
SEMINAL VESICLES	N	N	N	N	N	N	N	N	N	N
SKIN	N	N	N	N	N	N	N	N	N	N

(Report Continued)

Tabulated Animal Data

PROJECT ID: G-TI02 GROUP: Ti02 SEX: M DAYS: ALL
PAGE 16 FATES: SCHEDULED SACRIFICE

ANIMAL ID. NO:	497	498	499	500	501	502	503	504	505	506
URINARY BLADDER	N	N	N	N	N	N	N	N	N	N
PROSTATE	N	N	N	N	N	N	N	N	N	N
BONE (STERNUM)	N	N	N	N	N	N	N	N	N	N
BONE MARROW	N	N	N	N	N	N	N	N	N	N
NOSE	N	N	N	N	N	N	N	N	N	N

(End of Report)

VI. CORRELATION OF GROSS AND MICRO FINDINGS

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
OF IRON OXIDE AND GRAPHITE DUSTS
24 HOUR POST-EXPOSURE SACRIFICE

Correlation of Gross & Micro Findings

PROJECT ID: G-TI02 GROUP: CONTROL SEX: MALE DAYS: ALL
PAGE 1 FATES: ALL

ANIMAL NO: 400 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

ANIMAL NO: 401 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

ANIMAL NO: 402 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

ANIMAL NO: 403 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

>MANDIBULAR LYMPH NODE-RED NO COROLLARY CHANGE DETECTED

(Report Continued)

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
OF IRON OXIDE AND GRAPHITE DUSTS
24 HOUR POST-EXPOSURE SACRIFICE

Correlation of Gross & Micro Findings

PROJECT ID: G-TI02 GROUP: CONTROL SEX: MALE DAYS: ALL
PAGE 2 FATES: ALL

ANIMAL NO: 404 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

ANIMAL NO: 405 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

LIVER, MEDIAN LOBE-NODULE, 5X5X4MM, ROUND, BROWN, SOFT — LIVER- HEPATODIAPHRAGMATIC NODULE

ANIMAL NO: 406 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

NOTE: NOSE-EXTENSIVE BLOOD IN THE CAVITY, BUT NO INDICATION OF ORIGIN OF BLOOD. IT IS BELIEVED TO BE A RESULT OF NECROPSY.

(Report Continued)

Correlation of Gross & Micro Findings

PROJECT ID: G-TI02 GROUP: CONTROL SEX: MALE DAYS: ALL
PAGE 3 FATES: ALL

ANIMAL NO: 402 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

ANIMAL NO: 403 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

ANIMAL NO: 404 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

(Report Continued)

Correlation of Gross & Micro Findings

PROJECT ID: G-TI02 GROUP: MICRO260 SEX: MALE DAYS: ALL
PAGE 4 FATES: ALL

ANIMAL NO: 433 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

ANIMAL NO: 434 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

>LUNGS-DARK LUNGS- PIGMENTATION

ANIMAL NO: 435 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

ANIMAL NO: 436 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

>LUNG-DARK LUNGS- PIGMENTATION

(Report Continued)

Correlation of Gross & Micro Findings

PROJECT ID: G-TI02 GROUP: MICRO260 SEX: MALE DAYS: ALL
PAGE 5 FATES: ALL

ANIMAL NO: 437 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
>LIVER, MEDIAN LOBE-NODULE, 6X4X2MM, IRREGULAR	LIVER- HEPATODIAPHRAGMATIC NODULE

ANIMAL NO: 438 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
>LUNGS-DARK	LUNGS- PIGMENTATION

ANIMAL NO: 439 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

(Report Continued)

Correlation of Gross & Micro Findings

PROJECT ID: G-T002 GROUP: MICRO2+0 SEX: MALE DAYS: ALL
PAGE 6 FATES: ALL

ANIMAL NO: 440 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

ANIMAL NO: 441 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

LUNGS- DARK LUNGS- PIGMENTATION

ANIMAL NO: 442 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

(Report Continued)

Correlation of Gross & Micro Findings

PROJECT ID: G-T102 GROUP: MICRO&50 SEX: MALE DAYS: ALL
PAGE 7 FATES: ALL

ANIMAL NO: 465 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
>LUNGS-BLACK	LUNGS- PIGMENTATION
>KIDNEYS-MOTTLED	NO COROLLARY CHANGE DETECTED

ANIMAL NO: 466 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
>LUNGS-DARK	LUNGS- PIGMENTATION

ANIMAL NO: 467 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
>LUNG-DARK	LUNGS- PIGMENTATION

(Report Continued)

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
OF IRON OXIDE AND GRANITE DUSTS
24 HOUR POST-EXPOSURE SACRIFICE

Correlation of Gross & Micro Findings

PROJECT ID: G-T102 GROUP: MICRO650 SEX: MALE DAYS: ALL
PAGE 8 FATES: ALL

ANIMAL NO: 468 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
>PANCREAS-NODULE, 1X1X1MM, ROUND, RED	NO COROLLARY CHANGE DETECTED

ANIMAL NO: 469 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
>LUNGS-DARK	LUNGS- PIGMENTATION

ANIMAL NO: 470 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
>LUNGS-BLACK	LUNGS- PIGMENTATION

(Report Continued)

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
OF IRON OXIDE AND GRAPHITE DUSTS
24 HOUR POST-EXPOSURE SACRIFICE

Correlation of Gross & Micro Findings

PROJECT ID: G-F1G2 GROUP: MICRO&SO SEX: MALE DAYS: ALL
PAGE 9 RATES: ALL

ANIMAL NO: 4/1 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
>LUNG-DARK	LUNGS- PIGMENTATION

ANIMAL NO: 4/2 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
>TRIM: THYMUS-MOTTLED	THYMUS- HEMORRHAGE

ANIMAL NO: 4/3 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
>LUNG-DARK	LUNGS- PIGMENTATION

(Report Continued)

Correlation of Gross & Micro Findings

PROJECT ID: G-1102 GROUP: MICRO650 SEX: MALE DAYS: ALL
PAGE 10 FATES: ALL

ANIMAL NO: 474 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
LUNG-DARK	LUNGS- PIGMENTATION

(Report Continued)

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
OF IRON OXIDE AND GRAPHITE DUSTS
24 HOUR POST-EXPOSURE SACRIFICE

Correlation of Gross & Micro Findings

PROJECT ID: G-TI02 GROUP: Ti02 SEX: MALE DAYS: ALL
PAGE 11 FATES: ALL

ANIMAL NO: 497 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

LIVER, MEDIAN LOBE-NODULE, IRREGULAR LIVER- HEPATODIAPHRAGMATIC NODULE

ANIMAL NO: 498 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

ANIMAL NO: 499 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

ANIMAL NO: 500 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

(Report Continued)

Correlation of Gross & Micro Findings

PROJECT ID: G-TI02 GROUP: TiO2 SEX: MALE DAYS: ALL
PAGE 12 FATES: ALL

ANIMAL NO: 501 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

ANIMAL NO: 502 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

ANIMAL NO: 503 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

ANIMAL NO: 504 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

(Report Continued)

Correlation of Gross & Micro Findings

PROJECT ID: G-T102 GROUP: T102 SEX: MALE DAYS: ALL
PAGE 15 FATES: ALL

ANIMAL NO: 505 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

ANIMAL NO: 506 PATHOLOGIST: LHB
ANIMAL FATE: SCHEDULED SACRIFICE
DAYS ON TEST:15

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

(End of Report)

VII. QUALITY ASSURANCE STATEMENT

QUALITY ASSURANCE STATEMENT

This histopathology project has been inspected and audited by the quality assurance unit as required by the Good Laboratory Practice regulations promulgated by the U.S. Food and Drug Administration. Pathology Associates, Incorporated has a functioning and responsive quality assurance unit which reports directly to management. The following is a record of inspections/audits and their resulting reports:

Date of Inspection	Phase Inspected	Date Findings Reported to Management and Study Pathologist
*02-08-88	Tissue Trimming	02-08-88
*02-18-88	Processing/Embedding	02-18-88
*02-18-88	Microtomy	02-18-88
*02-19-88	Staining	02-19-88
*02-19-88	Coverslipping	02-19-88
*02-19-88	Labeling	02-19-88
**03-10-88	Individual Animal Data	03-11-88
**03-10-88	Data Entry	03-11-88
**03-10-88	Computer Validation	03-11-88
**03-11-88	Draft Pathology Report	03-11-88
**03-25-88	Final Pathology Report	03-25-88

*General Monthly Phase Inspection
** Inspection specific for Study Number

In concordance with the PAI Quality Assurance Division's Standard Operating Procedures, phase inspections are routinely conducted on a random basis at a minimum of monthly. Dates of inspection are reported for each study according to the most recent phase inspection conducted during that period.

Christine Stefanski — March 25, 1988

Director, Quality Assurance Unit — Date

Comparative Acute Inhalation Screen of Iron Oxide and Graphite Dusts (Four Day Exposure With Fourteen Day Recovery)(Protocol No. 22087000A217).

PAI Pathology Associates, Inc.

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Hyatt Park II
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PATHOLOGY REPORT
FOR

COMPARATIVE ACUTE INHALATION SCREEN OF IRON OXIDE AND GRAPHITE DUSTS FOUR DAY EXPOSURE WITH 14 DAY RECOVERY

PROTOCOL NO. 22087000A217

CONTRACT NO. DAAA15-85-D-0002

TABLE OF CONTENTS

I. PATHOLOGY REPORT

PATHOLOGY REPORT

COMPARATIVE ACUTE INHALATION SCREEN OF IRON OXIDE AND GRAPHITE DUSTS FOUR DAY EXPOSURE WITH 14 DAY RECOVERY

INTRODUCTION AND METHODS

Male F344 rats were divided into four groups of twenty each, and three of the groups were exposed to 100 mg/m^3 of natural graphite (Micro 650), synthetic graphite (Micro 260), or titanium dioxide (substituted for iron oxide) on four consecutive days, four hours/day. A control group of twenty rats was similarly exposed to air. Following the repeated exposure, ten rats per group were allowed to recover for one day after which time they were killed by carbon dioxide asphyxiation and necropsied. The body and organ weights and pathology findings from the 24 hour recovery groups were reported in an earlier pathology report. The remaining rats were allowed to recover for 14 days after the final exposure before they were killed and necropsied. At necropsy, the total body weight and the organ weights of adrenals, brain, heart, kidneys, liver, lung, and testes were recorded. The body and organ weights and pathology findings for the 14-day recovery groups are reported herein.

All organs and tissues required by contract were processed through paraffin, sectioned at approximately 6 μm, stained with hematoxylin and eosin, and examined microscopically. In addition to the required tissues, an attempt was made to evaluate peribronchial lymph nodes as well. An occasional tissue was lost during necropsy or processing and could not be examined microscopically.

RESULTS AND DISCUSSION

Gross Findings

The lungs of 6/10 rats exposed to natural graphite, 2/10 rats exposed to synthetic graphite, and 1/10 rat exposed to titanium dioxide were discolored or mottled, or had foci present. The gross lesions are presented in Section II. The remaining gross observations were miscellaneous, non-treatment-related changes. Body and organ weights are presented in Section III.

Gross lesions noted at necropsy or during trimming have been correlated with microscopic diagnoses, where possible, and are listed by animal in the CORRELATION OF GROSS AND MICROSCOPIC FINDINGS TABLE (Section VI). Correlative microscopic findings could not be determined for all gross lesions.

Microscopic Findings

Summary data are presented in the PROJECT SUMMARY TABLES (Section IV), which lists by group the number of animals having a given lesion. Microscopic findings for individual animals are presented by group in the TABULATED ANIMAL DATA TABLES (Section V). The codes used in the entries in these tables are explained in the Reports Code Table (Appendix 1), while topographic/morphologic abbreviations are explained in the Abbreviations List (Appendix 2).

Treatment-related changes were apparent in the lungs of all rats other than the controls. In each case, brown to black, isotropic pigment was present within macrophages in terminal airways and alveoli. No apparent free pigment (extracellular) as was seen in the 24-Hr. post-treatment groups was present in the lungs of these rats. Microscopically, the three types of pigment were indistinguishable from each other. However, in 6/10 rats exposed to synthetic graphite (Micro 260) and 8/10 rats exposed to natural graphite (Micro 650) pigment-laden macrophages tended to be aggregated in small groups (to a barely perceptible degree), more than in the rats exposed to titanium dioxide, and more than in any of the rats in the 24-Hr. post-exposure sacrifice groups. The only other changes in the lungs possibly associated with the treatment were one or two tiny (minimal) foci of epithelial hyperplasia in the alveoli and/or the terminal bronchioles of three rats exposed to synthetic graphite and one rat exposed to titanium dioxide. In no case were pigmented macrophages closely associated with the hyperplasia.

Focal hemorrhage of the lung was present in at least two rats from each group, to include the controls, and is believed to be associated with the carbon dioxide asphyxiation. Pigment was present in the peribronchial lymph nodes of 0/5, 1/8, and 1/6 rats in the Micro 260, Micro 650, and titanium dioxide exposed groups, respectively. In each case, the pigment present was very minimal (one or two macrophages).

The severity of the lung pigmentation was nearly identical within and between groups. It was graded as mild in all of the exposed rats. The reason why the pigmentation could not be seen grossly in all exposed rats is not known.

Several lesions commonly found in rats such as cardiomyopathy and nephropathy were present in rats in more than one dose group. In addition, thymic hemorrhage which is probably associated with euthanasia was present in several animals. These findings and all other lesions not mentioned are incidental findings and should not be associated with the test substances in any way.

CONCLUSIONS

Exposure of male F344 rats to natural graphite, synthetic graphite, or titanium dioxide by chamber inhalation for four days, four hours/day, followed by a 14-day recovery period resulted in lung changes in all exposed rats consisting of pigmentation (particulate deposition) within macrophages in alveoli and terminal airways. The pigment-laden macrophages were slightly more clumped together in six and eight rats in the synthetic and natural graphite-exposed groups, respectively, than in the titanium dioxide-exposed rats. Minimal epithelial hyperplasia of the terminal bronchioles and/or alveoli was present in three rats exposed to synthetic graphite and one rat exposed to titanium dioxide. No other significant differences were noted between 14-day recovery groups or between the 24-Hr. and 14-day groups.

Lucas H. Brennecke, D.V.M.
Diplomate, ACVP
March 24, 1988

APPENDIX I:
REPORTS CODE TABLE

TABLE II-1

GROSS NECROPSY FINDINGS

Dose Group	Animal No.	Lesion
Control		
	403	Mandibular lymph node - Red
	405	Liver - Nodule
Synthetic graphite		
	434	Lungs - Dark
	436	Lungs - Dark
	437	Liver - Nodule
	438	Lungs - Dark
	441	Lung - Dark
Natural graphite		
	465	Lungs - Black
		Kidneys - Mottled
	466	Lungs - Dark
	467	Lung - Dark
	468	Pancreas - Nodule
	469	Lungs - Dark
	470	Lungs - Black
	471	Lung - Dark
(Trim)	472	Thymus - Mottled
	473	Lung - Dark
	474	Lung - Dark
Titanium dioxide		
	497	Liver - Nodule

III. ORGAN AND BODY WEIGHTS

Table III-1
Organ and Body Weights (grams)

Animal No.	Group	Body Wt.	Adrenals	Brain	Heart	Kidneys	Liver	Lungs	Testes
415	Control	313.3	.057	1.77	.85	2.27	11.22	1.13	2.82
418	Control	307.5	.035	1.88	.90	2.22	10.92	1.26	3.02
419	Control	302.9	.035	1.84	.86	2.32	11.50	1.21	2.97
420	Control	289.1	.037	1.84	.78	2.12	9.88	1.24	2.91
421	Control	293.4	.037	1.84	.71	2.04	9.16	1.12	2.82
422	Control	276.5	.035	1.81	.79	1.95	9.95	1.09	2.99
423	Control	306.2	.045	1.94	.98	2.48	12.61	1.38	3.11
424	Control	311.0	.038	1.87	.79	2.24	11.77	1.28	2.90
425	Control	314.8	.048	1.81	.82	2.30	11.32	1.15	3.01
426	Control	303.7	.042	1.81	.86	2.27	12.81	.95	2.89
Mean:		301.8	.041	1.84	.83	2.22	11.11	1.18	2.94
449	260	280.4	.043	1.86	.91	2.28	10.82	1.14	2.89
450	260	297.5	.035	1.86	.84	2.22	10.32	1.07	2.72
451	260	306.8	.043	1.84	.83	2.17	10.73	1.27	2.91
452	260	306.4	.038	1.93	.80	2.25	11.01	1.37	2.50
453	260	306.5	.048	1.90	.79	2.21	10.42	1.23	2.91
454	260	306.0	.044	1.82	.88	2.22	12.26	1.11	2.89
455	260	313.9	.042	1.88	.85	2.23	12.08	1.31	2.92
456	260	317.7	.036	1.87	.87	2.27	11.61	1.26	3.00
457	260	277.8	.038	1.81	.75	2.11	9.81	1.09	2.73
458	260	324.8	.047	1.92	.89	2.48	12.93	1.36	2.97
Mean:		303.78	.041	1.87	.84	2.24	11.20	1.22	2.84
481	650	281.3	.035	1.81	.82	2.13	9.95	1.17	2.58
482	650	307.3	.041	1.89	.87	2.28	10.61	1.42	3.02
483	650	308.1	.029	1.88	.81	2.21	10.08	1.17	2.98
484	650	301.8	.041	1.86	.80	2.26	10.33	1.24	3.06
485	650	288.9	.041	1.84	.86	2.02	10.72	1.28	2.90
486	650	314.4	.040	1.88	.92	2.38	11.45	1.23	3.11
487	650	297.8	.034	1.87	.78	2.07	10.18	1.26	2.75
488	650	293.3	.037	1.89	.73	2.13	9.49	1.27	3.02
489	650	309.6	.044	1.89	.76	2.07	11.11	1.03	2.85
490	650	315.8	.032	1.88	.88	2.47	12.04	1.49	2.95
Mean:		301.8	.037	1.87	.82	2.20	10.60	1.26	2.92

Table III-1 (Continued)

Animal No.	Group	Body Wt.	Adrenals	Brain	Heart	Kidneys	Liver	Lungs	Testes
513	Ti. Diox.	318.8	.040	1.87	.86	2.19	10.63	1.08	3.16
514	Ti. Diox.	310.1	.035	1.85	.84	2.15	10.68	1.31	2.99
515	Ti. Diox.	317.3	.043	1.92	.77	2.12	10.42	1.40	3.04
516	Ti. Diox.	307.6	.038	1.86	.91	2.35	10.89	1.41	3.13
517	Ti. Diox.	300.0	.033	1.86	.77	1.97	10.49	1.25	2.96
518	Ti. Diox.	321.1	.038	1.99	.83	2.10	12.03	1.25	3.02
519	Ti. Diox.	314.3	.039	1.85	.94	2.50	11.68	1.20	3.19
520	Ti. Diox.	302.6	.049	1.81	1.00	2.21	10.99	1.16	2.92
521	Ti. Diox.	310.6	.045	1.88	.86	2.39	11.13	1.23	2.92
522	Ti. Diox.	298.7	.042	1.91	.81	2.22	10.47	1.31	2.85
Mean:		310.1	.040	1.88	.86	2.22	10.94	1.26	3.02

IV. SUMMARY TABLE OF MICROSCOPIC FINDINGS BY GROUP

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
OF IRON OXIDE AND GRAPHITE DUSTS
24 HOUR POST-EXPOSURE SACRIFICE

Reports Code Table

N	Tissues within normal histological limits
A	Autolysis precluding adequate evaluation
P	Paired organ missing
U	Tissues unavailable/unsuitable for evaluation
S	Tissues not applicable to animal
*	Tissues not examined/not required by protocol

1	minimal
2	mild
3	moderate
4	marked
)	focal
]	diffuse
)	multifocal
P	Present
B	Neoplasm, Benign
M	Neoplasm, Malignant without Metastasis
C	Neoplasm, Malignant with Metastasis
X	Metastatic Site (+)
-	No data entered

APPENDIX II:
ABBREVIATIONS LIST

ABBREVIATIONS LIST

Epithelium	Epith.
Hyperplasia	Hyperpl.
Perivascular	PV
Terminal	Term.

II. GROSS NECROPSY FINDINGS

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
OF IRON OXIDE AND GRAPHITE DUSTS
14 DAY POST EXPOSURE SACRIFICE

Project Summary Table

SUMMARY: Incidence of NEOPLASTIC and NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: G-14PE
PAGE 1

FATES: ALL
DAYS: ALL
SEX: MALE

GROUP:		CONTROL	MICRO260	MICRO650	T102
NUMBER OF ANIMALS:		10	10	10	10
BRAIN	# Ex	10	10	10	10
SCIATIC NERVE	# Ex	10	10	9	10
SPINAL CORD	# Ex	10	10	10	10
ZYMBAL'S GLAND	# Ex	10	8	10	9
SALIVARY GLAND	# Ex	10	10	10	10
PANCREAS	# Ex	10	10	10	10
MANDIBULAR LYMPH NODE	# Ex	10	10	10	10
THYMUS	# Ex	10	10	10	10
HEMORRHAGE		4	4	2	2
TRACHEA	# Ex	10	10	10	10
LARYNX	# Ex	10	10	10	10
THYROIDS	# Ex	10	10	10	10
PARATHYROIDS	# Ex	10	10	9	9
PITUITARY	# Ex	9	10	10	10
PARS DISTALIS-HYPERPLASIA		1	0	0	0

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
OF IRON OXIDE AND GRAPHITE DUSTS
14 DAY POST EXPOSURE SACRIFICE

Project Summary Table

SUMMARY: Incidence of NEOPLASTIC and NON-NEOPLASTIC Microscopic Findings

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PROJECT ID. NO: G-14PE
PAGE 1

FATES: ALL
DAYS: ALL
SEX: MALE

GROUP:		CONTROL	MICRO260	MICRO650	TiO2
NUMBER OF ANIMALS:		10	10	10	10
		#	#	#	#
BRAIN	# Ex	10	10	10	10
SCIATIC NERVE	# Ex	10	10	9	10
SPINAL CORD	# Ex	10	10	10	10
ZYMBAL'S GLAND	# Ex	10	8	10	9
SALIVARY GLAND	# Ex	10	10	10	10
PANCREAS	# Ex	10	10	10	10
MANDIBULAR LYMPH NODE	# Ex	10	10	10	10
THYMUS	# Ex	10	10	10	10
HEMORRHAGE		4	4	2	2
TRACHEA	# Ex	10	10	10	10
LARYNX	# Ex	10	10	10	10
THYROIDS	# Ex	10	10	10	10
PARATHYROIDS	# Ex	10	10	9	9
PITUITARY	# Ex	9	10	10	10
PARS DISTALIS-HYPERPLASIA		1	0	0	0

Project Summary Table

SUMMARY: Incidence of NEOPLASTIC and NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: G-14PE FATES: ALL
PAGE 2 DAYS: ALL SEX: MALE

GROUP:		CONTROL	MICRO260	MICRO650	TiO2
NUMBER OF ANIMALS:		10	10	10	10
		#	#	#	#
LUNGS	# Ex	10	10	10	10
HEMORRHAGE		2	4	3	3
PIGMENTATION		0	10	10	10
INTERSTITIUM-INFLAMMATION		0	0	1	0
PV-INFILTRATE, LYMPHOCYTIC		0	1	0	0
ALVEOLAR EPITH-HYPERPLASIA		0	2	0	0
TERM BRONCHIOLE EPITH-HYPERPL		0	1	0	1
BRONCHIAL LYMPH NODE	# Ex	6	5	8	6
HEMORRHAGE		0	1	0	0
CONGESTION		0	0	1	0
PIGMENTATION		0	0	1	1
EYE	# Ex	10	10	10	10
SCLERA-MINERALIZATION		2	1	1	0
CORNEA-DEGENERATION		0	0	1	0
FORESTOMACH	# Ex	10	10	10	10
GLANDULAR STOMACH	# Ex	10	10	10	10
ESOPHAGUS	# Ex	10	10	10	10
DUODENUM	# Ex	10	10	10	10
COLON	# Ex	10	10	10	10
MESENTERIC LYMPH NODE	# Ex	10	10	10	10
CONGESTION		1	0	0	0
LIVER	# Ex	10	10	10	10
VACUOLIZATION, CYTOPLASMIC		0	1	1	0

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
OF IRON OXIDE AND GRAPHITE DUSTS
14 DAY POST EXPOSURE SACRIFICE

Project Summary Table

SUMMARY: Incidence of NEOPLASTIC and NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: G-14PE FATES: ALL
PAGE 3 DAYS: ALL SEX: MALE

GROUP:		CONTROL	MICRO26D	MICRO65D	TiO2
NUMBER OF ANIMALS:		10	10	10	10
		#	#	#	#
LIVER	# Ex	10	10	10	10
HEMORRHAGE		0	0	0	1
SPLEEN	# Ex	10	10	10	10
HEART	# Ex	10	10	10	10
CARDIOMYOPATHY		6	3	1	2
KIDNEYS	# Ex	10	10	10	10
NEPHROPATHY		1	1	1	2
HEMORRHAGE		1	0	0	0
SKELETAL MUSCLE	# Ex	10	10	10	10
ADRENAL CORTEX	# Ex	10	10	10	10
HEMORRHAGE		0	1	0	0
ADRENAL MEDULLA	# Ex	9	10	10	10
TESTES	# Ex	10	10	10	10
ATROPHY		0	1	0	0
SEMINAL VESICLES	# Ex	10	10	10	10
SKIN	# Ex	10	10	10	10
URINARY BLADDER	# Ex	10	10	10	10
PROSTATE	# Ex	10	10	10	10

Project Summary Table

SUMMARY: Incidence of NEOPLASTIC and NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: G-14PE
PAGE 4

FATES: ALL
DAYS: ALL
SEX: MALE

GROUP:		CONTROL	MICRO260	MICRO6S0	Ti02
NUMBER OF ANIMALS:		10	10	10	10
		#	#	#	#
BONE (STERNUM)	# Ex	10	10	10	10
BONE MARROW	# Ex	10	10	10	10
NOSE	# Ex	10	10	10	10
HEMORRHAGE		1	0	0	2

(Report Continued)

Project Summary Table

SUMMARY: Incidence of NEOPLASTIC and NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: G-14PE
PAGE 5

FATES: ALL
DAYS: ALL
SEX: MALE

GROUP:	CONTROL	MICRO260	MICRO650	TiO2
NUMBER OF ANIMALS:	10	10	10	10
	#	#	#	#
OTHER TISSUES AND LESIONS:				
PANCREAS-ACCESSORY SPLEEN	0	1	0	0
MESENTERY, FAT-NECROSIS	0	0	1	0

(End of Report)

V. TABULATED INDIVIDUAL ANIMAL TABLE

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
OF IRON OXIDE AND GRAPHITE DUSTS
14 DAY POST EXPOSURE SACRIFICE

Tabulated Animal Data

PROJECT ID: G-14PE GROUP: CONTROL SEX: M DAYS: ALL
PAGE 1 FATES: ALL

ANIMAL ID. NO:	415	418	419	420	421	422	423	424	425	426
BRAIN	N	N	N	N	N	N	N	N	N	N
SCIATIC NERVE	N	N	N	N	N	N	N	N	N	N
SPINAL CORD	N	N	N	N	N	N	N	N	N	N
ZYMBAL'S GLAND	N	N	N	N	N	N	N	N	N	N
SALIVARY GLAND	N	N	N	N	N	N	N	N	N	N
PANCREAS	N	N	N	N	N	N	N	N	N	N
MANDIBULAR LYMPH NODE	N	N	N	N	N	N	N	N	N	N
THYMUS	N	N		N		N	N			N
HEMORRHAGE	-	-	1	-	1	-	-	1	2	-
TRACHEA	N	N	N	N	N	N	N	N	N	N
LARYNX	N	N	N	N	N	N	N	N	N	N
THYROIDS	N	N	N	N	N	N	N	N	N	N

Tabulated Animal Data

PROJECT ID: G-14PE GROUP: CONTROL SEX: M DAYS: ALL
PAGE 2 FATES: ALL

ANIMAL ID. NO:	415	418	419	420	421	422	423	424	425	426
PARATHYROIDS	N	N	N	N	N	N	N	N	N	N
PITUITARY	N	N		N	N	N	U	N	N	N
PARS DISTALIS-HYPERPLASIA	-	-	(1)	-	-	-	-	-	-	-
LUNGS	N	N	N	N	N		N	N	N	
HEMORRHAGE	-	-	-	-	-	(1)	-	-	-	(1)
BRONCHIAL LYMPH NODE	U	N	N	N	N	N	U	N	U	U
EYE	N	N	N		N	N		N	N	N
SCLERA-MINERALIZATION	-	-	-	(1)	-	-	(1)	-	-	-
FORESTOMACH	N	N	N	N	N	N	N	N	N	N
GLANDULAR STOMACH	N	N	N	N	N	N	N	N	N	N
ESOPHAGUS	N	N	N	N	N	N	N	N	N	N
DUODENUM	N	N	N	N	N	N	N	N	N	N
COLON	N	N	N	N	N	N	N	N	N	N

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
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14 DAY POST EXPOSURE SACRIFICE

Tabulated Animal Data

PROJECT ID: G-14PE GROUP: CONTROL SEX: M DAYS: ALL
PAGE 3 FATES: ALL

ANIMAL ID. NO:	415	418	419	420	421	422	423	424	425	426
MESENTERIC LYMPH NODE	N		N	N	N	N	N	N	N	N
CONGESTION	-	2	-	-	-	-	-	-	-	-
LIVER	N	N	N	N	N	N	N	N	N	N
SPLEEN	N	N	N	N	N	N	N	N	N	N
HEART	N	N			N					N
CARDIOMYOPATHY	-	-	1	2	-	1	2	2	1	-
KIDNEYS	N		N	N	N	N	N		N	N
NEPHROPATHY	-	-	-	-	-	-	-	1	-	-
HEMORRHAGE	-	(2)	-	-	-	-	-	-	-	-
SKELETAL MUSCLE	N	N	N	N	N	N	N	N	N	N
ADRENAL CORTEX	N	N	N	N	N	N	N	N	N	N
ADRENAL MEDULLA	N	N	U	N	N	N	N	N	N	N
TESTES	N	N	N	N	N	N	N	N	N	N
SEMINAL VESICLES	N	N	N	N	N	N	N	N	N	N

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
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14 DAY POST EXPOSURE SACRIFICE

Tabulated Animal Data

PROJECT ID: G-14PE GROUP: CONTROL SEX: M DAYS: ALL
PAGE 4 FATES: ALL

ANIMAL ID. NO:	415	418	419	420	421	422	423	424	425	426
SKIN	N	N	N	N	N	N	N	N	N	N
URINARY BLADDER	N	N	N	N	N	N	N	N	N	N
PROSTATE	N	N	N	N	N	N	N	N	N	N
BONE (STERNUM)	N	N	N	N	N	N	N	N	N	N
BONE MARROW	N	N	N	N	N	N	N	N	N	N
NOSE	N	N	N	N	N	N	N	N		N
HEMORRHAGE	-	-	-	-	-	-	-	-	2	-

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
OF IRON OXIDE AND GRAPHITE DUSTS
14 DAY POST EXPOSURE SACRIFICE

Tabulated Animal Data

PROJECT ID: G-14PE GROUP: CONTROL SEX: M DAYS: ALL
PAGE 5 FATES: ALL

ANIMAL ID. NO:	415	418	419	420	421	422	423	424	425	426

OTHER TISSUES AND LESIONS:

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
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14 DAY POST EXPOSURE SACRIFICE

Tabulated Animal Data

PROJECT ID: G-14PE GROUP: MICRO260 SEX: M DAYS: ALL
PAGE 6 FATES: ALL

ANIMAL ID. NO:	449	450	451	452	453	454	455	456	457	458
BRAIN	N	N	N	N	N	N	N	N	N	N
SCIATIC NERVE	N	N	N	N	N	N	N	N	N	N
SPINAL CORD	N	N	N	N	N	N	N	N	N	N
ZYMBAL'S GLAND	N	N	N	N	N	U	U	N	N	N
SALIVARY GLAND	N	N	N	N	N	N	N	N	N	N
PANCREAS	N	N	N	N	N	N	N	N	N	N
MANDIBULAR LYMPH NODE	N	N	N	N	N	N	N	N	N	N
THYMUS			N	N		N	N	N		N
HEMORRHAGE	2	2	-	-	2	-	-	-	2	-
TRACHEA	N	N	N	N	N	N	N	N	N	N
LARYNX	N	N	N	N	N	N	N	N	N	N
THYROIDS	N	N	N	N	N	N	N	N	N	N

Tabulated Animal Data

PROJECT ID: G-14PE GROUP: MICR0260 SEX: M DAYS: ALL
PAGE 7 FATES: ALL

ANIMAL ID. NO:	449	450	451	452	453	454	455	456	457	458
PARATHYROIDS	N	N	N	N	N	N	N	N	N	N
PITUITARY	N	N	N	N	N	N	N	N	N	N
LUNGS										
HEMORRHAGE	(1)	-	-	-	(1)	-	(1)	(1)	-	-
PIGMENTATION	2	2	2	2	2	2	2	2	2	2
PV-INFILTRATE, LYMPHOCYTIC	(1)	-	-	-	-	-	-	-	-	-
ALVEOLAR EPITH-HYPERPLASIA	(1)	-	-	-	-	-	-	-	(1)	-
TERM BRONCHIOLE EPITH-HYPERPL	-	(1)	-	-	-	-	-	-	-	-
BRONCHIAL LYMPH NODE	U	U	N	N	U	U	N		U	N
HEMORRHAGE	-	-	-	-	-	-	-	2	-	-
EYE	N	N	N	N	N	N		N	N	N
SCLERA-MINERALIZATION	-	-	-	-	-	-	(1)	-	-	-
FORESTOMACH	N	N	N	N	N	N	N	N	N	N
GLANDULAR STOMACH	N	N	N	N	N	N	N	N	N	N
ESOPHAGUS	N	N	N	N	N	N	N	N	N	N
DUODENUM	N	N	N	N	N	N	N	N	N	N

Tabulated Animal Data

PROJECT ID: G-14PE GROUP: MICR0260 SEX: M DAYS: ALL
PAGE 8 FATES: ALL

ANIMAL ID. NO:	449	450	451	452	453	454	455	456	457	458
COLON	N	N	N	N	N	N	N	N	N	N
MESENTERIC LYMPH NODE	N	N	N	N	N	N	N	N	N	N
LIVER	N	N	N	N	N	N	N		N	N
VACUOLIZATION, CYTOPLASMIC	-	-	-	-	-	-	-	(2)	-	-
SPLEEN	N	N	N	N	N	N	N	N	N	N
HEART	N	N	N	N			N		N	N
CARDIOMYOPATHY	-	-	-	-	1	1	-	1	-	-
KIDNEYS	N	N	N		N	N	N	N	N	N
NEPHROPATHY	-	-	-	1	-	-	-	-	-	-
SKELETAL MUSCLE	N	N	N	N	N	N	N	N	N	N
ADRENAL CORTEX	N	N	N	N	N	N	N	N	N	
HEMORRHAGE	-	-	-	-	-	-	-	-	-	(2)
ADRENAL MEDULLA	N	N	N	N	N	N	N	N	N	N
TESTES	N	N	N		N	N	N	N	N	N
ATROPHY	-	-	-	2	-	-	-	-	-	-

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
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Tabulated Animal Data

PROJECT ID: G-14PE GROUP: MICRO260 SEX: M DAYS: ALL
PAGE 9 FATES: ALL

ANIMAL ID. NO:	449	450	451	452	453	454	455	456	457	458
SEMINAL VESICLES	N	N	N	N	N	N	N	N	N	N
SKIN	N	N	N	N	N	N	N	N	N	N
URINARY BLADDER	N	N	N	N	N	N	N	N	N	N
PROSTATE	N	N	N	N	N	N	N	N	N	N
BONE (STERNUM)	N	N	N	N	N	N	N	N	N	N
BONE MARROW	N	N	N	N	N	N	N	N	N	N
NOSE	N	N	N	N	N	N	N	N	N	N

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
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Tabulated Animal Data

PROJECT ID: G-14PE GROUP: MICRO260 SEX: M DAYS: ALL
PAGE 10 FATES: ALL

ANIMAL ID. NO:	449	450	451	452	453	454	455	456	457	458
OTHER TISSUES AND LESIONS:										
PANCREAS-ACCESSORY SPLEEN	-	-	-	-	P	-	-	-	-	-

Tabulated Animal Data

PROJECT ID: G-14PE GROUP: MICRO650 SEX: M DAYS: ALL
PAGE 11 FATES: ALL

ANIMAL ID. NO:	481	482	483	484	485	486	487	488	489	490
BRAIN	N	N	N	N	N	N	N	N	N	N
SCIATIC NERVE	N	U	N	N	N	N	N	N	N	N
SPINAL CORD	N	N	N	N	N	N	N	N	N	N
ZYMBAL'S GLAND	N	N	N	N	N	N	N	N	N	N
SALIVARY GLAND	N	N	N	N	N	N	N	N	N	N
PANCREAS	N	N	N	N	N	N	N	N	N	N
MANDIBULAR LYMPH NODE	N	N	N	N	N	N	N	N	N	N
THYMUS	N	N	N		N		N	N	N	N
HEMORRHAGE	-	-	-	2	-	1	-	-	-	-
TRACHEA	N	N	N	N	N	N	N	N	N	N
LARYNX	N	N	N	N	N	N	N	N	N	N
THYROIDS	N	N	N	N	N	N	N	N	N	N

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
OF IRON OXIDE AND GRAPHITE DUSTS
14 DAY POST EXPOSURE SACRIFICE

Tabulated Animal Data

PROJECT ID: G-14PE GROUP: MICRO650 SEX: M DAYS: ALL
PAGE 12 FATES: ALL

ANIMAL ID. NO:	481	482	483	484	485	486	487	488	489	490
PARATHYROIDS	N	N	N	U	N	N	N	N	N	N
PITUITARY	N	N	N	N	N	N	N	N	N	N
LUNGS										
HEMORRHAGE	-	-	(1)	-	-	-	-	-	(2)	(1)
PIGMENTATION	2	2	2	2	2	2	2	2	2	2
INTERSTITIUM-INFLAMMATION	-	-	-	-	-	-	-	(1)	-	-
BRONCHIAL LYMPH NODE	N	N	N	N	N	U			U	N
CONGESTION	-	-	-	-	-	-	3	-	-	-
PIGMENTATION	-	-	-	-	-	-	-	1	-	-
EYE	N	N	N	N		N	N	N		N
SCLERA-MINERALIZATION	-	-	-	-	(1)	-	-	-	-	-
CORNEA-DEGENERATION	-	-	-	-	-	-	-	-	(1)	-
FORESTOMACH	N	N	N	N	N	N	N	N	N	N
GLANDULAR STOMACH	N	N	N	N	N	N	N	N	N	N
ESOPHAGUS	N	N	N	N	N	N	N	N	N	N
DUODENUM	N	N	N	N	N	N	N	N	N	N

Tabulated Animal Data

PROJECT ID: G-14PE GROUP: MICRO650 SEX: M DAYS: ALL
PAGE 13 FATES: ALL

ANIMAL ID. NO:	481	482	483	484	485	486	487	488	489	490
COLON	N	N	N	N	N	N	N	N	N	N
MESENTERIC LYMPH NODE	N	N	N	N	N	N	N	N	N	N
LIVER	N	N	N	N	N	N	N	N		N
VACUOLIZATION, CYTOPLASMIC	-	-	-	-	-	-	-	-	(1)	-
SPLEEN	N	N	N	N	N	N	N	N	N	N
HEART	N	N	N	N	N		N	N	N	N
CARDIOMYOPATHY	-	-	-	-	-	2	-	-	-	-
KIDNEYS	N	N	N	N	N	N	N	N		N
NEPHROPATHY	-	-	-	-	-	-	-	-	1	-
SKELETAL MUSCLE	N	N	N	N	N	N	N	N	N	N
ADRENAL CORTEX	N	N	N	N	N	N	N	N	N	N
ADRENAL MEDULLA	N	N	N	N	N	N	N	N	N	N
TESTES	N	N	N	N	N	N	N	N	N	N
SEMINAL VESICLES	N	N	N	N	N	N	N	N	N	N

PATHOLOGY ASSOCIATES, INC.
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Tabulated Animal Data

PROJECT ID: G-14PE GROUP: MICRO650 SEX: M DAYS: ALL
PAGE 14 FATES: ALL

ANIMAL ID. NO:	481	482	483	484	485	486	487	488	489	490
SKIN	N	N	N	N	N	N	N	N	N	N
URINARY BLADDER	N	N	N	N	N	N	N	N	N	N
PROSTATE	N	N	N	N	N	N	N	N	N	N
BONE (STERNUM)	N	N	N	N	N	N	N	N	N	N
BONE MARROW	N	N	N	N	N	N	N	N	N	N
NOSE	N	N	N	N	N	N	N	N	N	N

PATHOLOGY ASSOCIATES, INC.
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Tabulated Animal Data

PROJECT ID: G-14PE GROUP: MICRO650 SEX: M DAYS: ALL
PAGE 15 FATES: ALL

ANIMAL ID. NO:	481	482	483	484	485	486	487	488	489	490
OTHER TISSUES AND LESIONS:										
MESENTERY, FAT-NECROSIS	-	-	-	-	-	-	-	-	-	(2)

Tabulated Animal Data

PROJECT ID: G-14PE GROUP: TiO2 SEX: M DAYS: ALL
PAGE 16 FATES: ALL

ANIMAL ID. NO:	513	514	515	516	517	518	519	520	521	522
BRAIN	N	N	N	N	N	N	N	N	N	N
SCIATIC NERVE	N	N	N	N	N	N	N	N	N	N
SPINAL CORD	N	N	N	N	N	N	N	N	N	N
ZYMBAL'S GLAND	N	N	N	N	U	N	N	N	N	N
SALIVARY GLAND	N	N	N	N	N	N	N	N	N	N
PANCREAS	N	N	N	N	N	N	N	N	N	N
MANDIBULAR LYMPH NODE	N	N	N	N	N	N	N	N	N	N
THYMUS	N	N	N	N	N	N		N		N
HEMORRHAGE	-	-	-	-	-	-	1	-	2	-
TRACHEA	N	N	N	N	N	N	N	N	N	N
LARYNX	N	N	N	N	N	N	N	N	N	N
THYROIDS	N	N	N	N	N	N	N	N	N	N

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
OF IRON OXIDE AND GRAPHITE DUSTS
14 DAY POST EXPOSURE SACRIFICE

Tabulated Animal Data

PROJECT ID: G-14PE GROUP: TiO2 SEX: M DAYS: ALL
PAGE 17 FATES: ALL

ANIMAL ID. NO:	513	514	515	516	517	518	519	520	521	522
PARATHYROIDS	U	N	N	N	N	N	N	N	N	N
PITUITARY	N	N	N	N	N	N	N	N	N	N
LUNGS										
HEMORRHAGE	-	-	-	-	-	(1)	-	(1)	-	(2)
PIGMENTATION	2	2	2	2	2	2	2	2	2	2
TERM BRONCHIOLE EPITH-HYPERPL	-	-	-	(1)	-	-	-	-	-	-
BRONCHIAL LYMPH NODE	N	N	N		N	U	U	U	N	U
PIGMENTATION	-	-	-	1	-	-	-	-	-	-
EYE	N	N	N	N	N	N	N	N	N	N
FORESTOMACH	N	N	N	N	N	N	N	N	N	N
GLANDULAR STOMACH	N	N	N	N	N	N	N	N	N	N
ESOPHAGUS	N	N	N	N	N	N	N	N	N	N
DUODENUM	N	N	N	N	N	N	N	N	N	N
COLON	N	N	N	N	N	N	N	N	N	N

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
OF IRON OXIDE AND GRAPHITE DUSTS
14 DAY POST EXPOSURE SACRIFICE

Tabulated Animal Data

PROJECT ID: G-14PE GROUP: TiO2 SEX: M DAYS: ALL
PAGE 18 FATES: ALL

ANIMAL ID. NO:	513	514	515	516	517	518	519	520	521	522
MESENTERIC LYMPH NODE	N	N	N	N	N	N	N	N	N	N
LIVER	N	N	N	N	N	N	N	N	N	
HEMORRHAGE	-	-	-	-	-	-	-	-	-	(2)
SPLEEN	N	N	N	N	N	N	N	N	N	N
HEART		N	N	N	N	N		N	N	N
CARDIOMYOPATHY	1	-	-	-	-	-	1	-	-	-
KIDNEYS	N	N		N	N	N		N	N	N
NEPHROPATHY	-	-	1	-	-	-	1	-	-	-
SKELETAL MUSCLE	N	N	N	N	N	N	N	N	N	N
ADRENAL CORTEX	N	N	N	N	N	N	N	N	N	N
ADRENAL MEDULLA	N	N	N	N	N	N	N	N	N	N
TESTES	N	N	N	N	N	N	N	N	N	N
SEMINAL VESICLES	N	N	N	N	N	N	N	N	N	N
SKIN	N	N	N	N	N	N	N	N	N	N

Tabulated Animal Data

PROJECT ID: G-14PE GROUP: TiO2 SEX: M DAYS: ALL
PAGE 19 FATES: ALL

ANIMAL ID. NO:	513	514	515	516	517	518	519	520	521	522
URINARY BLADDER	N	N	N	N	N	N	N	N	N	N
PROSTATE	N	N	N	N	N	N	N	N	N	N
BONE (STERNUM)	N	N	N	N	N	N	N	N	N	N
BONE MARROW	N	N	N	N	N	N	N	N	N	N
NOSE	N	N	N	N	N		N	N		N
HEMORRHAGE	-	-	-	-	-	1	-	-	3	-

Tabulated Animal Data

PROJECT ID: G-14PE GROUP: TiO2 SEX: M DAYS: ALL
PAGE 20 FATES: ALL

ANIMAL ID. NO:	513	514	515	516	517	518	519	520	521	522

OTHER TISSUES AND LESIONS:

VI. CORRELATION OF GROSS AND MICRO FINDINGS

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
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14 DAY POST EXPOSURE SACRIFICE

Correlation of Gross & Micro Findings

PROJECT ID: G-14PE GROUP: CONTROL SEX: MALE DAYS: ALL
PAGE 1 FATES: ALL

ANIMAL NO: 415 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

ANIMAL NO: 418 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

ANIMAL NO: 419 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

ANIMAL NO: 420 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

Correlation of Gross & Micro Findings

PROJECT ID: G-14PE GROUP: CONTROL SEX: MALE DAYS: ALL
PAGE 2 FATES: ALL

ANIMAL NO: 421 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
>KIDNEY, BILATERAL-FOCI, MULTIPLE, RED	NO COROLLARY CHANGE DETECTED

ANIMAL NO: 422 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:

ANIMAL NO: 423 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:

ANIMAL NO: 424 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:

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Correlation of Gross & Micro Findings

PROJECT ID: G-14PE GROUP: CONTROL SEX: MALE DAYS: ALL
PAGE 3 FATES: ALL

ANIMAL NO: 425 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

ANIMAL NO: 426 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
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Correlation of Gross & Micro Findings

PROJECT ID: G-14PE GROUP: MICRO260 SEX: MALE DAYS: ALL
PAGE 4 FATES: ALL

ANIMAL NO: 449 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

ANIMAL NO: 450 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

ANIMAL NO: 451 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

>

COMMENT: LUNG-AS COMPARED TO THE 24-HR SACRIFICE, MORE PIGMENT (IN MACROPHAGES) IS CLUMPED TOGETHER IN TERMINAL BRONCHIOLES.

Correlation of Gross & Micro Findings

PROJECT ID: G-14PE GROUP: MICRO260 SEX: MALE DAYS: ALL
PAGE 5 FATES: ALL

ANIMAL NO: 452 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
>LUNG-MOTTLED	LUNGS- PIGMENTATION

ANIMAL NO: 453 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
>PANCREAS-NODULE, 2X2X1MM, ROUND, RED	PANCREAS-ACCESSORY SPLEEN
>	COMMENT: LUNG-AS COMPARED TO THE 24-HOUR SACRIFICE, MORE PIGMENT (IN MACROPHAGES) IS CLUMPED TOGETHER IN TERMINAL BRONCHIOLES.

ANIMAL NO: 454 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
>	COMMENT: LUNG-AS COMPARED TO THE 24-HOUR SACRIFICE, MORE PIGMENT (IN MACROPHAGES) IS CLUMPED TOGETHER IN TERMINAL BRONCHIOLES.

Correlation of Gross & Micro Findings

PROJECT ID: G-14PE GROUP: MICRO260 SEX: MALE DAYS: ALL
PAGE 6 FATES: ALL

ANIMAL NO: 455 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

ANIMAL NO: 456 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

>THYMUS-FOCI, 1X1X1MM, ROUND, DARK RED. NO COROLLARY CHANGE DETECTED

> COMMENT: LUNG-AS COMPARED TO THE 24-HOUR SACRIFICE, MORE PIGMENT (IN MACROPHAGES) IS CLUMPED TOGETHER IN TERMINAL BRONCHIOLES.

ANIMAL NO: 457 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

> COMMENT: LUNG-AS COMPARED TO THE 24-HOUR SACRIFICE, MORE PIGMENT (IN MACROPHAGES) IS CLUMPED TOGETHER IN TERMINAL BRONCHIOLES.

PATHOLOGY ASSOCIATES, INC.
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Correlation of Gross & Micro Findings

PROJECT ID: G-14PE GROUP: MICRO260 SEX: MALE DAYS: ALL
PAGE 1 FATES: ALL

ANIMAL NO: 458 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE

DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
>LUNG-GREY	LUNGS- PIGMENTATION; COMMENT: LUNG-AS COMPARED TO THE 24-HOUR SACRIFICE, MORE PIGMENT (IN MACROPHAGES) IS CLUMPED TOGETHER IN TERMINAL BRONCHIOLES.

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
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Correlation of Gross & Micro Findings

PROJECT ID: G-14PE GROUP: MICRO-50 SEX: MALE DAYS: ALL
PAGE 8 FATES: ALL

ANIMAL NO: 481 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
>LUNG-GREY	LUNGS- PIGMENTATION; COMMENT: LUNG PIGMENT IS IN LARGER CLUMPS (MOSTLY IN TERMINAL BRONCHIOLES) THAN IN THE 24 HOUR STUDY. PIGMENT ALSO IN BALT.

ANIMAL NO: 482 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
>LUNG-MOTTLED	LUNGS- PIGMENTATION; COMMENT: LUNG PIGMENT IS IN LARGER CLUMPS (MOSTLY IN TERMINAL BRONCHIOLES) THAN IN THE 24 HOUR STUDY. PIGMENT ALSO IN BALT.

ANIMAL NO: 483 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:

Correlation of Gross & Micro Findings

PROJECT ID: G-14PE GROUP: MICRO650 SEX: MALE DAYS: ALL
PAGE 9 RATES: ALL

ANIMAL NO: 483 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
>LUNG, ALL LOBES-FOCI, MULTIPLE, RED	LUNGS- HEMORRHAGE; COMMENT: LUNG PIGMENT IS IN LARGER CLUMPS (MOSTLY IN TERMINAL BRONCHIOLES) THAN IN THE 24 HOUR STUDY. PIGMENT ALSO IN BALT.

ANIMAL NO: 484 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
>	COMMENT: LUNG PIGMENT IS IN LARGER CLUMPS (MOSTLY IN TERMINAL BRONCHIOLES) THAN IN THE 24 HOUR STUDY. PIGMENT ALSO IN BALT.

ANIMAL NO: 485 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:

PATHOLOGY ASSOCIATES, INC.
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Correlation of Gross & Micro Findings

PROJECT ID: G-14PE GROUP: MICR0650 SEX: MALE DAYS: ALL
PAGE 10 FATES: ALL

ANIMAL NO: 486 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE

DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

ANIMAL NO: 487 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE

DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

>LUNG-DARK

LUNGS- PIGMENTATION; COMMENT:
LUNG PIGMENT IS IN LARGER CLUMPS
(MOSTLY IN TERMINAL BRONCHIOLES)
THAN IN THE 24 HOUR STUDY.
PIGMENT ALSO IN BALT.

>BRONCHIAL LYMPH NODE-ENLARGED,
4X3X2MM, OVAL, BROWN

BRONCHIAL LYMPH NODE- CONGESTION

ANIMAL NO: 488 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE

DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

>

COMMENT: LUNG PIGMENT IS IN
LARGER CLUMPS (MOSTLY IN TERMINAL
BRONCHIOLES) THAN IN THE 24 HOUR
STUDY. PIGMENT ALSO IN BALT.

PATHOLOGY ASSOCIATES, INC.
COMPARATIVE ACUTE INHALATION SCREEN
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Correlation of Gross & Micro Findings

PROJECT ID: G-14PE GROUP: MICRO650 SEX: MALE DAYS: ALL
PAGE 11 FATES: ALL

ANIMAL NO: 489 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
>LUNG, ALL LOBES-FOCI, MULTIPLE, ROUND, RED	LUNGS- HEMORRHAGE; COMMENT: LUNG PIGMENT IS IN LARGER CLUMPS (MOSTLY IN TERMINAL BRONCHIOLES) THAN IN THE 24 HOUR STUDY. PIGMENT ALSO IN BALT.

ANIMAL NO: 490 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:13

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
>MESENTERY, FAT-NODULE, 3X2X1MM, ROUND, YELLOW	MESENTERY, FAT NECROSIS
>LUNG-GREY	LUNGS- PIGMENTATION; COMMENT: LUNG PIGMENT IS IN LARGER CLUMPS (MOSTLY IN TERMINAL BRONCHIOLES) THAN IN THE 24 HOUR STUDY. PIGMENT ALSO IN BALT.

PATHOLOGY ASSOCIATES, INC.
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14 DAY POST EXPOSURE SACRIFICE

Correlation of Gross & Micro Findings

PROJECT ID: G-14FE GROUP: 1102 SEX: MALE DAYS: ALL
PAGE 12 FATES: ALL

ANIMAL NO: 513 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

ANIMAL NO: 514 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

COMMENT: LUNG-PIGMENT-LADEN MACROPHAGES PRESENT IN SOME LARGER BRONCHIOLES, BUT IT MAY BE ARTIFACTUAL.

ANIMAL NO: 515 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

Correlation of Gross & Micro Findings

PROJECT ID: G-14PE GROUP: Ti02 SEX: MALE DAYS: ALL
PAGE 13 FATES: ALL

ANIMAL NO: 516 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:

ANIMAL NO: 517 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:

ANIMAL NO: 518 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:
LUNG, ALL LOBES-FOCI, MULTIPLE, ROUND, RED	LUNGS- HEMORRHAGE

ANIMAL NO: 519 PATHOLOGIST: LHB
ANIMAL FATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD:	RELATED HISTOPATHOLOGY:

PATHOLOGY ASSOCIATES, INC.
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Correlation of Gross & Micro Findings

PROJECT ID: G-14PE GROUP: T102 SEX: MALE DAYS: ALL
PAGE 14 RATES: ALL

ANIMAL NO: 520 PATHOLOGIST: LHB
ANIMAL RATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

ANIMAL NO: 521 PATHOLOGIST: LHB
ANIMAL RATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

> COMMENT: LUNG-PIGMENTED MACROPHAGES PRESENT IN SOME SMALL BRONCHIOLES.

ANIMAL NO: 522 PATHOLOGIST: LHB
ANIMAL RATE: TERMINAL SACRIFICE
DAYS ON TEST:18

REFERENCE TO NECROPSY RECORD: RELATED HISTOPATHOLOGY:

VII. QUALITY ASSURANCE STATEMENT

QUALITY ASSURANCE STATEMENT

This histopathology project has been inspected and audited by the quality assurance unit as required by the Good Laboratory Practice regulations promulgated by the U.S. Food and Drug Administration. Pathology Associates, Incorporated has a functioning and responsive quality assurance unit which reports directly to management. The following is a record of inspections/audits and their resulting reports:

Date of Inspection	Phase Inspected	Date Findings Reported to Management and Study Pathologist
*02-08-88	Tissue Trimming	02-08-88
*02-18-88	Processing/Embedding	02-18-88
*02-18-88	Microtomy	02-18-88
*02-19-88	Staining	02-19-88
*02-19-88	Coverslipping	02-19-88
*02-19-88	Labeling	02-19-88
**02-25-88	Individual Animal Data	02-25-88
**02-25-88	Data Entry	02-25-88
**02-25-88	Computer Validation	02-25-88
**02-25-88	Draft Pathology Report	02-25-88
**03-09-88	Second Draft Gross to Microscopic Tables	03-09-88
**03-25-88	Final Pathology Report	03-25-88

*General Monthly Phase Inspection
** Inspection specific for Study Number

In concordance with the PAI Quality Assurance Division's Standard Operating Procedures, phase inspections are routinely conducted on a random basis at a minimum of monthly. Dates of inspection are reported for each study according to the most recent phase inspection conducted during that period.

Director, Quality Assurance Unit

March 25, 1988
Date

Comparative Acute Inhalation Screen of Iron Oxide and Graphite Dusts (Four Day Exposure With One Day Recovery)(Protocol No. 22087000A217).